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AGING PROCESSES IN THE OVARIES OF MICE BELONGING TO STRAINS DIFFERING IN THE INCIDENCE OF MAMMARY CARCINOMA

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I. THE FATE OF FOLLICLES AND CORPORA LUTEA

THE PRESENT investigations on aging processes in the ovaries of various strains of mice represent a part of a larger series in which the changes taking place in old age in the ovaries, mammary glands, vagina, uterus and the thyroid and adrenal glands were studied mainly in mice but to some extent also in guinea pigs. In addition the skeletal system has been investigated from this point of view by Martin and Ruth Silberberg.

MATERIAL

We distinguish four periods in the life of the mouse as follows: period 1 (from birth up to 9 months of age), period 2 (from 10 to 14 months), period 3 (from 15 to 19 months) and period 4 (20 months and older).

Mice of the various strains were distributed in these four periods as follows:

PERIOD 1.—Strain C: 20 nonbreeding and 4 breeding mice. Strain AKA: 34 nonbreeding and 2 breeding mice. Strain CBA: 17 nonbreeding mice; 1 breeding mouse. Strain C57 black: 53 nonbreeding and 5 breeding mice. Strain Old Buffalo: 2 nonbreeding mice. Strain New Buffalo: 3 nonbreeding mice. Strain C3H: 19 nonbreeding and 7 breeding mice. Strain D: 20 nonbreeding and 6 breeding mice. Strain A: 45 nonbreeding and 3 breeding mice. In period 1 the total numbers were 213 nonbreeding and 28 breeding mice, altogether 241 mice.

PERIOD 2.—Strain C: 3 nonbreeding and 4 breeding mice. Strain AKA: 11 nonbreeding and 2 breeding mice. Strain CBA: 9 nonbreeding and 5 breeding mice. Strain C57 black: 28 nonbreeding and 14 breeding mice. Strain Old Buffalo: 17 nonbreeding and 2 breeding mice. Strain New Buffalo: 2 nonbreeding and 10 breeding mice. Strain C3H: 17 nonbreeding and 14 breeding mice. Strain D: 49 nonbreeding and 14 breeding mice. Strain A: 44 nonbreeding and 29 breeding mice. In period 2 the total numbers were 180 nonbreeding and 94 breeding mice, althogether 274 mice.

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The ovaries, on the microscopic examination of which these investigations are based, were collected by H. T. Blumenthal, Marian Moskop Kirtz and V. Suntzeff, in the course of various experiments carried out in this laboratory.

Period 3.—Strain C: 3 breeding mice. Strain AKA: 5 nonbreeding and 3 breeding mice. Strain CBA: 2 nonbreeding and 8 breeding mice. Strain C57 black: 12 nonbreeding and 12 breeding mice. Strain Old Buffalo: 2 nonbreeding and 4 breeding mice. Strain New Buffalo: 14 nonbreeding and 5 breeding mice. Strain C3H: 3 nonbreeding and 4 breeding mice. Strain D: 12 nonbreeding and 4 breeding mice. Strain A: 16 nonbreeding and 10 breeding mice. In period 3 the total numbers were 66 nonbreeding and 53 breeding mice, altogether 119 mice.

Period 4.—Strain AKA: 4 nonbreeding mice, 20 to 23 months of age; 1 breeding mouse, 24 months of age. Strain CBA: 8 nonbreeding mice, 21 to 31 months of age; 6 breeding mice, 20 to 28 months of age. Strain C57 black: 9 nonbreeding mice, 20 to 25½ months of age; 1 breeding mouse, 26 months of age. Strain Old Buffalo: 4 nonbreeding mice, 24 to 29 months of age; 8 breeding mice, 20 to 28 months of age. Strain New Buffalo: 4 nonbreeding mice, 20 to 28 months of age; 2 breeding mice, 23 months of age. Strain C3H: 1 nonbreeding mouse, 20 months of age. Strain D: 4 nonbreeding mice, 20 to 22 months of age; 1 breeding mouse, 22 months of age. Strain A: 7 nonbreeding mice, 20 to 24½ months of age; 1 breeding mouse, 21 months of age. In period 4 the total numbers were 41 nonbreeding and 20 breeding mice, altogether 61 animals.

The numbers of mice of all periods combined were 500 nonbreeding and 195 breeding mice, altogether 695 animals. We designate as breeding mice all those female mice which had one or more litters in their life.

OBSERVATIONS

In this first section I shall study the fate of the follicles and corpora lutea with advancing age. In the second section I shall study the interstitial gland, the ducts developing in the ovary and the changes which they undergo with advancing age. I shall record only the more significant findings in the various strains and refer to some others in the discussion of the observations.

Strain C.—*Follicles.*—Period 1. In the center of atretic follicles there were groups of yellow cells, presumably representing modified granulosa cells. During the process of atresia connective tissue cells may penetrate through the membrana granulosa and surround the egg; in 1 instance a granulosa cell seemed to penetrate between the segments of an egg.

Period 2. Large follicles were present in almost all the mice. In one atretic follicle hemorrhage was noted around the egg. Blood vessels could grow into the granulosa of follicles during the process of atresia.

Period 3. Large follicles had developed in all 3 mice.

Corpora Lutea.—Period 1. Only exceptionally hyalinization or gelatinization was seen in a corpus luteum. Degeneration usually occurred by way of vacuolation of the lutein cells and by ingrowth of connective tissue, and following this, the shrunken corpora lutea could be replaced by interstitial gland tissue. This mode of degeneration was the usual one.

Period 2. Vacuolation of the corpora lutea could begin in the center of these structures and progress toward the periphery.

Period 3. In all 3 breeding mice preserved corpora lutea, and in 2 of them even young corpora lutea, were found. Typical hyalinization was lacking, but a softening and solution of the central parts of the corpus luteum could occur, a process which was perhaps related to gelatinization.

STRAIN AKA.—*Follicles.*—Period 1. During atresia the membrana granulosa might become vacuolar or a part of it might become dissolved; connective tissue cells might penetrate through the granulosa to the egg, and in some instances they entered the egg.

Period 2. Some enlarged follicles were seen in 1 mouse. In another mouse one end of the ovary consisted of dilated capillaries.

Period 3. In 1 mouse very large follicles with central hemorrhage were present. In some of the ovaries primordial follicles were noted. In the majority of the ovaries there were full-sized follicles.

Period 4. In the youngest mouse very large follicles were seen, but usually the follicles reached only a small size; then degeneration of the granulosa set in. But also in the oldest mouse very large and hemorrhagic follicles were found.

Corpora Lutea.—Period 1. Some mitoses occurred in luteal cells of young corpora lutea in a mouse 2 months, 6 days of age. After these structures had reached full development, they underwent degenerative vacuolation and then were invaded by connective tissue and also by interstitial gland tissue; in the end shrinking took place. Hyaline changes were seen in the corpora lutea only exceptionally.

Period 2. In 5 mice bluish-staining young, as well as fully mature preserved, corpora lutea were present. But in the majority of these animals there were in addition corpora lutea in various stages of hyalinization. In some ovaries almost all the corpora lutea were hyalinized. Also hyaline arteries were seen. In 1 of the 2 breeding mice there were two hyaline remnants of corpora lutea, and here also the walls of the arteries were largely hyaline.

Period 3. A hyaline artery was seen in the ovary. In 2 breeding mice there were young bluish corpora lutea, in some of which beginning of hyalinization seemed to be noticeable. Older corpora lutea were to a variable degree or even entirely hyalinized. The hyalinization seemed to begin around arteries; there was also hyalin around arteries in various parts of the ovaries. In 1 mouse some corpora lutea underwent vacuolar degeneration.

Period 4. In the majority of these animals there were shrunken corpora lutea, remnants of corpora lutea and hyaline or vacuolar corpora lutea. In 3 younger nonbreeding mice some remnants of vacuolar or shrunken corpora lutea were found; there occurred also hyaline and partly gelatinous corpora lutea. In the single breeding mouse a great part of the ovary consisted of gelatinous corpora lutea in which the nuclei had been lost. Such necrotic gelatinous areas could be surrounded by foreign body giant cells.

STRAIN CBA.—*Follicles.*—Period 1. In the breeding mouse a whole strand of primordial follicles was seen in tissue adjoining the medulla. In atretic follicles the centrally situated cells, probably granulosa cells, could be changed into vacuolar yellow cells and a thin layer of connective tissue could grow to the inner margin of the membrana granulosa lining the central cavity.

Period 2. In two mice some hemorrhage was seen in follicles; in one of these mice a very large follicle was hemorrhagic. In several mice the granulosa of atretic follicles carried yellow pigment.

Period 3. Among the older mice of this period, 2 mice had large follicles, and one of these had also very large follicles. In 3 animals no follicles were seen. Also primordial follicles occurred in this group of mice. Among the younger mice, no follicles or only atretic ones were seen in 2 mice, and in a third one large follicles were lacking.

Period 4. No follicles were found in the 10 oldest mice. Also in the younger mice of this group no large follicles had developed.

Corpora Lutea.—Period 1. Hyalinization was not observed in the corpora lutea of this period.

Period 2. Some young, bluish-staining corpora lutea were noted, but mostly the corpora lutea were older, and red-staining vacuolation, but no hyalinization, appeared in these structures. Some corpora lutea seemed to have been replaced by interstitial gland tissue. When during the early stages of degeneration the circulation was slowed down in a corpus luteum, leukocytes could collect in the central portion of the corpus luteum.

Period 3. Mostly no corpora lutea or only remnants of corpora lutea were found.

Period 4. In 3 nonbreeding mice remnants of corpora lutea or connective tissue-invaded degenerating corpora lutea were present; in 1 nonbreeding mouse red-staining corpora lutea were found; in all the other animals corpora lutea were lacking. There were no hyaline corpora lutea.

STRAIN C57 BLACK.—*Follicles.*—Period 1. The large majority of the mice had large follicles. During atresia the granulosa cells changed into vacuolar yellow cells in some ovaries; it appeared that these yellow cells had phagocytosed red blood corpuscles. Enlarged follicles, some of which were hyperemic or hemorrhagic, occurred in a few mice.

Period 2. In almost all the mice full-sized follicles were present, and in 14 of 42 animals mature or just ruptured follicles were seen. In some of the nonruptured follicles hemorrhages developed, probably having their origin in capillaries growing into the granulosa. Capillaries were seen to invade the granulosa of just ruptured follicles as well as that of nonruptured follicles. The nonruptured follicles could give rise to the formation of pseudo lutein bodies.

Period 3. In a number of mice large follicles were absent; in others, between 15 and 17 months old, mature or just ruptured follicles were seen. In several mice very large, hemorrhagic follicles were noted; hemorrhages occurred also in atretic follicles. Characteristic of strain C57 black mice was the tendency of granulosa cells in degenerating follicles in some animals to form syncytia; at the same time the nuclei could assume a rosette-like arrangement.

Period 4. In 2 mice the ovaries were infiltrated with, and partly replaced by, leukemic lymphocytes. In one half of the remaining mice some follicles reached full development; in the other half the follicles remained small or medium sized. Nonatretic, as well as atretic, follicles could be hemorrhagic, and in atretic hemorrhagic follicles vacuolar granulosa cells seemed to take up blood pigment.

Corpora Lutea.—Period 1. No hyaline corpora lutea were seen.

Period 2. There was no hyalinization. Some pseudo corpora lutea developed after capillaries grew into the membrana granulosa of nonruptured follicles.

Period 3. Among the older mice of this period, between the ages of 17 and 20 months, several animals had good corpora lutea, and in one of them young corpora lutea were seen. In 1 mouse hyalinization of corpora lutea was noted. Also a hyaline artery and a small hyaline area were found in the center of the ovary in 1 mouse. In 2 animals, lacking corpora lutea, hyaline material surrounding capillaries extended from the hilus upward. Hyalinization around vessels could occur probably independently of corpora lutea.

Period 4. There were no preserved corpora lutea during this period, nor were there degenerating vacuolar or hyalinized-gelatinized corpora lutea; but in a

mouse 20½ months of age and weighing 22 Gm. two fairly young corpora lutea with some vacuolation and hemorrhage in the center were seen.

STRAIN OLD BUFFALO.—*Follicles.*—Period 2. In the large majority of mice large follicles were present, and in 4 animals mature or just ruptured follicles were seen. In some large follicles hemorrhages occurred. In an atretic follicle some granulosa cells moved between the segments of a segmented egg.

Period 3. In the large majority of the animals large follicles were lacking. In a medium-sized follicle there was some hemorrhage.

Period 4. In 5 mice, 26 months of age or older either no follicles were seen or follicles reached at most small to medium size. In mice younger than 26 months, large follicles or even follicles which approached maturity occurred. A primordial follicle was found in the interstitial gland.

Corpora Lutea.—Period 2. Regression took place by shrinking and vacuolation of lutein cells. Only in 1 mouse were hyaline corpora lutea seen, and here hyalinization began around blood vessels. There was also ingrowth of connective tissue in two hyaline corpora lutea.

Period 3. In 1 nonbreeding mouse lutein-like tissue of uncertain nature was seen in which colloid and vacuolar changes took place in some cells. In the 4 breeding mice preserved corpora lutea, and in 1 mouse almost 20 months old even young corpora lutea were present. In the oldest mouse there were some strands of hyaline tissue.

Period 4. Among the 4 nonbreeding mice, varying in age between 24 and 29 months, no corpora lutea were found in 2 animals. In the remaining 2 there were large masses of gelatinous corpora lutea. In 2 breeding mice, 28 months old, there seemed to be preserved corpora lutea. In 2 breeding mice 26 months old, there were gelatinous corpora lutea or corpus luteum-like tissue. In leukemic mice about 20 months of age the greater part of the corpora lutea, including partly hyalinized or gelatinized corpora lutea, had been destroyed by lymphocytes. In the 2 remaining animals, 20 to 21 months old, preserved mature or vacuolar corpora lutea were noted.

STRAIN NEW BUFFALO.—*Follicles.*—In periods 1 and 2 the follicles reached their full development in most cases; however, in a mouse with low weight, the ovaries were hypotypical.

In period 3 all variations were seen from ovaries with fully developed follicles to ovaries devoid of all follicles or containing only atretic follicles. Some follicles became cystic, and hemorrhages were seen in some very large follicles, but also in other follicles, including atretic ones.

In period 4 conditions were similar to those observed in period 3. Hemorrhages occurred here also, and it seemed that hemorrhages in the follicles could give rise to yellow pigment in the granulosa cells.

Corpora Lutea.—Periods 1 and 2. In some mice regression of corpora lutea took place by vacuolation, but in 4 animals of period 2 hyaline-gelatinous corpora lutea were seen.

Period 3. In about one half of the mice no corpora lutea or only doubtful ones were found; but even in 19 to 20 month old mice new corpora lutea could develop. In a number of mice, especially in the older ones in this period, gelatinization occurred in corpora lutea. In the younger mice corpora lutea in general were better preserved than in the older mice of this period.

Period 4. In mice as old as 28 months, as well as in younger mice, mostly remnants of corpora lutea were found. In some of the animals the corpora lutea

underwent vacuolation, connective tissue ingrowth and shrinking, while in others they underwent hyalinization. Some hyaline clumps which were observed probably represented also remnants of hyaline corpora lutea.

STRAIN C3H.—*Follicles.*—Period 1. In the large majority of mice in this period, and also in period 2, large follicles were found; also mature follicles were seen in some cases. During atresia in periods 1 and 2 the granulosa cells could undergo vacuolation and acquire yellow pigment; also the theca cells could become vacuolated. Connective tissue was seen to invade the membrana granulosa.

Period 2. In atretic follicles both theca and granulosa cells could be vacuolated, but they could be distinguished from each other by the fact that the granulosa cells were the larger. These two tissues could be separated by a hyaline band.

Period 3. In all the mice some large follicles were seen and in 2 mice there were very large or cystic follicles. Hemorrhagic follicles, in which the hemorrhage had destroyed the granulosa, also occurred.

Period 4. In the single mouse examined at this period no large follicles and only a single small follicle were seen.

Corpora Lutea.—Period 1. In the nonbreeding mice corpora lutea in various stages of development were found. Connective tissue penetrated into some of the older corpora lutea which were in process of regression; also remnants of such regressing corpora lutea were seen. In the breeding mice the findings were similar; but there was 1 mouse, 9 months of age and weighing 39 Gm., which showed a peculiar condition. In one ovary there were a number of well developed corpora lutea, but no new corpora lutea were found. In the other ovary there was a blood clot. At one end this ovary was seen to be connected with the fallopian tube. The blood clot was lined with several layers of cells, the nuclei of which were unusually large and contained prominent nucleoli. Some of these cells had two nuclei. These cellular layers around the blood clot represented syncytial formations which were similar to those observed in ovaries of the guinea pig, where in the center of such layers in several instances well preserved embryonal structures were found. Furthermore, in the guinea pig some of the syncytial giant cells penetrated into the surrounding tissue. Similarly in this mouse the corresponding cells migrated into the wall of the fallopian tube, in which a nest of such cells was seen. In addition to these syncytial structures, folds of the germinal epithelium which was connected with the surface of the ovary surrounded the blood clot. This condition might represent either an extrauterine pregnancy in which the fertilized ovum had fixed itself to the fallopian tube or parthenogenetic development of an egg, which would correspond to the findings in the ovaries of guinea pigs. However, there was no pregnancy in the uterus, and it appears more probable, therefore, that in this mouse one has to deal with parthenogenetic development of an egg which attached itself to a place where fimbria and germinal epithelium of the ovary were connected with an abortive extrauterine pregnancy. The blood clot owed its origin presumably to a hemorrhage which was induced by erosion of blood vessels. This resulted when embryonal placental lining cells migrated through the blood vessel walls into neighboring areas. Also in guinea pigs hemorrhages could be caused in this way.

Period 2. Only in some of the nonbreeding mice did hyalinization of the corpora lutea occur; but in these animals this process could progress so far that the greater part of the ovary consisted of gelatinous material, in which vessels with necrotic walls were seen. In only 3 of the 14 breeding mice did hyalinization of the corpora lutea occur, and in 2 of these animals hyaline material surrounded the arteries.

Side by side with the processes of hyalinization there was development of new corpora lutea in some ovaries. In 1 animal regression of a corpus luteum took place by vacuolation.

Period 3. In the oldest of the 3 nonbreeding mice no corpora lutea were seen; in the remaining 2 animals gelatinization of corpora lutea occurred to different degrees. Various adjoining corpora lutea were seen to coalesce, with gelatinization of a great part of the ovary resulting. At the same time new corpora lutea could form, which then proceeded toward maturation. Some hyalinization occurred around small arteries. Some corpora lutea, however, degenerated by vacuolation.

Period 4. In the only mouse present at this period, a nonbreeding animal, 20 months of age and weighing 29 Gm., gelatinized as well as better preserved corpora lutea were seen.

STRAIN D.—*Follicles.*—Period 1. The membrana granulosa in atretic follicles could be vacuolar, and it contained either yellow pigment or no pigment.

Period 2. Large follicles were seen in the large majority of the animals. In a number of mice mature or recently ruptured follicles were present, and in other animals larger than normal follicles occurred. As usual a great part of the granulosa remained preserved during the early stages of atresia, but it could undergo vacuolation and a layer of connective tissue could cover the inner surface of the granulosa; in 1 animal the ingrowing connective tissue destroyed the eggs in the centers of atretic follicles. In later stages of atresia the greater portion of the granulosa might be destroyed and connective tissue might fill the central cavity.

Period 3. In 9 of the 12 nonbreeding mice no full-sized follicles were seen, and in 2 of these 9 mice follicles were lacking altogether. In 1 mouse a larger than normal follicle was observed, and in another mouse there was blood in the central cavity. In still another animal the greater portion of the ovary had been destroyed by a blood clot. In the breeding mice there were ovaries in which no follicles were preserved or in which follicles were entirely lacking; but in 1 mouse follicles were well developed; also a hemorrhagic follicle was seen in an ovary.

Period 4. No follicles were seen except in 1 nonbreeding mouse, 22 months old, in which some small preserved follicles and a primordial follicle were noted. The presence of follicles in older mice is not, therefore, characteristic of high mammary tumor strains and does not distinguish them from low tumor strains.

Corpora Lutea.—Period 1. In this period and also in period 2 the mice in which corpora lutea were lacking were usually of low weight; this of course does not apply to young mice in which, on account of their age, ovulation had not yet taken place. The corpora lutea which had formed in the other mice were free of hyalinization. Also in the breeding mice hyalinization was lacking.

Period 2. Mitoses appeared in preserved corpora lutea, although rarely. After the stage of maturation has been reached, as a rule processes of hyalinization set in; but in some animals hyalinization was delayed or did not occur; instead, ingrowth of connective tissue and shrinking of the corpora lutea took place. However, vacuolation of the corpora lutea was rare as compared with hyalinization and gelatinization. Hyalinization could also affect the arteries in corpora lutea. Occasionally a deposit of calcium salts was found in gelatinized areas. In 1 animal, in a certain place in the corpus luteum some corpus luteum cells seemed to send pseudopodia out into adjoining gelatinous areas. This picture suggested the beginning of a process of invasion in which the gelatinous material might be substituted by the adjoining preserved corpus luteum tissue. Hyaline rings were seen surrounding capillaries in a corpus luteum. Hyalinization may first appear

around the vessels and then extend to the lutein cells, in which hyaline droplets become visible. Connective tissue and blood vessels also may grow into gelatinized areas. Interstitial glands may take part in the invasion of gelatinized corpora lutea.

Period 3. Hyalinization and gelatinization of corpora lutea were pronounced, and hyalinization seemed to begin in the periphery of the corpora lutea. In some peripheral areas of ovaries and in places underneath the germinal epithelium there could still be seen some better preserved corpus luteum tissue. In the hyalinized and gelatinized tissue some walls of arteries were hyalinized. New corpora lutea still developed. In 5 mice no preserved corpus luteum tissue was found, the ovaries consisting largely of gelatinous material. On the other hand, in 2 mice, 15 and 16 months of age, there were mature or shrunken but not hyalinized or gelatinized corpora lutea, into which connective tissue seemed to have grown. In the 2 oldest mice, 19 months old, among 4 breeders some vacuolated corpora lutea were seen. In mice about 16 months old hyalinization of corpora lutea was lacking; instead there were preserved young corpora lutea.

Period 4. In all the 4 nonbreeding mice, 20 to 22 months of age, small preserved corpora lutea were present besides larger gelatinized corpora lutea or remnants of gelatinized corpora lutea. In 2 the corpus luteum tissue had some tumor-like characteristics; it contained large nuclei and seemed to be slowly growing. In another mouse what appeared as young corpus luteum tissue consisted apparently of coiled ducts, and this tissue likewise had in some respects a tumor-like appearance. Parts of some corpora lutea were preserved, while other parts were hyalinized. In the breeding female there were mainly corpora lutea in different stages of gelatinization, and solution processes also had occurred. In gelatinized corpora lutea there were hyalinized vessel walls in which in places some calcium salts had been deposited.

STRAIN A.—*Follicles.*—Period 1. Only in a small number of mice were large follicles lacking, and these animals had a low weight.

Period 2. Very large and mature follicles occurred similar to those found in period 1. There were in addition the ordinary large follicles. In 4 mice cystically dilated, hemorrhagic follicles were seen.

Period 3. (*a*) Mice 17 to 19 months of age: There was only 1 animal in which the follicles were not larger than of small to medium size. In several mice very large and even cystic follicles were seen, and in 2 animals some follicles had just ruptured. In some of the mice primordial follicles were present, such as had been observed also in period 1. (*b*) Mice 15 to 17 months of age: In almost all these mice large follicles were present in addition to very large and cystic, hemorrhagic follicles in some of the mice. In a large follicle in which the membrana granulosa had been lost in the upper segment, the granulosa cells elsewhere had enlarged around the egg; they became vacuolar, and some of them seemed to phagocytose a part of the egg. In several other follicles some granulosa cells seemed to contain phagocytosed material. Other granulosa cells could disintegrate into colloid substance. In some atretic follicles the theca interna enlarged and became vacuolated, while the granulosa cells enlarged and became like corpus luteum cells. Thus pseudo corpora lutea developed.

Period 4. In 3 of 8 mice no full-sized follicles developed, but almost all the animals had some cystic or very large follicles with or without hemorrhagic changes.

Corpora Lutea.—Period 1. Corpora lutea were lacking not only in some young mice, which presumably had not yet ovulated, but also in several older mice of this period, especially if their weight was below 20 Gm. Regression of the corpora

lutea occurred, as a rule, by vacuolation and connective tissue ingrowth. In 3 mice hyaline corpora lutea were found. An artery in a hyaline-gelatinous corpus luteum was surrounded by hyaline material. In 2 animals, 5 and 6 months of age, hyaline corpora lutea were not seen, but hyaline material surrounded some arteries.

Period 2. In a large majority of the mice hyaline corpora lutea, showing various stages of hyalinization or gelatinization, were present. But also young, well preserved corpora lutea were found together with hyaline corpora lutea in a number of animals. The young corpora lutea were especially frequent in the younger mice of this period. In the hyaline corpora lutea connective tissue could disappear in the periphery, while the more central gelatinized part could be more or less organized and replaced by connective tissue, or in other instances the gelatinous areas could soften and liquefy still further. Connective tissue grew also into the margin of the preserved corpora lutea. In the periphery of one corpus luteum there was a zone containing clumps of blood, which were surrounded by foreign body giant cells. Hyalin appeared also around a vessel. Besides the hyaline degeneration of corpora lutea, other types of regression were seen in some of the corpora lutea.

Period 3. (*a*) Mice aged 17 to 19 months: In the majority of ovaries hyaline-gelatinous material predominated; in such areas the connective tissue could either still be present or be dissolved. But in some ovaries a number of red-staining mature corpora lutea or some of their remnants could still be seen. In other ovaries even partly preserved corpora lutea were absent. In 1 mouse there were structures which may have been luteinized follicles (pseudo corpora lutea). In one ovary small, not yet gelatinized corpora lutea were preserved, but very cellular connective tissue strands invaded these structures and destroyed them to a considerable extent. Also vacuolar degenerated corpora lutea were seen. Some pinkish-staining areas consisting of vacuolar cells, which contained yellow blood pigment, probably represented theca interna cells of atretic follicles. In hyaline-gelatinous material, present in the ovary and composed of conglomerated, degenerated corpora lutea, a completely necrotic artery containing some bluish calcium deposits in its walls was seen. In other gelatinous corpora lutea a few preserved capillaries could still be present. (*b*) Mice aged 15 to 17 months: Here again, much hyalinization and gelatinization were found in the ovaries, but a number of good corpora lutea and even of rather young corpora lutea occurred; in such a structure a mitotic figure was seen in a corpus luteum cell. There were also large areas of vacuolated cells, which represented remnants of degenerated vacuolar corpora lutea. In places it seemed that also interstitial gland was in part hyalinized, In 3 mice belonging to this age group no corpora lutea were found.

Period 4. In 3 nonbreeding mice either no corpus luteum was present or there were remnants of corpora lutea, and in one ovary there was probably a luteinized follicle representing a pseudo lutein body. In the remaining 5 mice there were corpora lutea in various stages of gelatinization, but there were also some vacuolated corpora lutea or even some areas of well preserved corpus luteum tissue. In a breeding mouse there was a largely gelatinized remnant of corpus luteum tissue; besides, the greater part of the ovary was hyalinized or gelatinized, with hyalinization affecting also the stroma. In the gelatinized material cavity formation could take place.

COMMENT

Some of the conclusions which may be drawn from the changes observed in ovaries of the various strains will now be set forth under appropriate headings.

Development of Follicles in the Different Periods of Life.—In periods 1 and 2, as a rule, the follicles of the ovaries of the large majority of mice of all the strains develop in a normal manner and some of these reach full size. However, in very young mice, those below the age of 2 months, or in mice which have just ovulated, or even in somewhat older mice which are underweight, and occasionally also in other mice, fully developed follicles may be lacking. In strain D in a number of breeding mice large follicles were not seen in period 4, and besides there was a reduction in the number of follicles in some of the animals. There was no noticeable difference between high and low rate mammary cancer strains as far as the development of follicles is concerned in the first year of life. In period 3 some decline takes place in the development of the follicles, but even in this period follicles may still be well developed, as, for instance, in strains C and AKA. In strain C57 a considerable number of mice had large follicles, but in others they did not reach full size or the number of follicles was reduced. In strain C3H all mice had large follicles, while in strain D large follicles were lacking in the large majority of mice and in 2 mice follicles were lacking altogether; also in strain Old Buffalo large follicles, as a rule, were lacking. Thus in period 3 there is a definite decrease in the number of mice in which large follicles have developed but there is a considerable difference in the findings in different strains without regard to whether they are high or low mammary cancer strains.

In period 4 the decline in the development of the follicles is more pronounced than in period 3. Thus during period 4 in strain AKA follicular atresia set in usually prematurely. In strain Old Buffalo no follicles or only very small ones were seen in the oldest mice of this period, although in the younger animals large follicles could develop. In strain CBA no follicles were seen in the ovaries of the oldest mice, but even in the younger animals follicles did not develop in a normal manner. In strain C57 the result was better in period 4. In about one half of the mice large follicles developed in the first half of this period, whereas in the second half only small or medium-sized follicles were seen. In strain D no follicles were found except in 1 mouse, in which undeveloped follicles were present.

Presence of Mature or Recently Ruptured Follicles.—In periods 1 and 2 mature or just ruptured follicles could be seen in nonbreeding as well as in breeding mice. In animals 5 weeks of age or younger ovulation usually did not seem to occur. In period 3 mature or just ruptured follicles appeared only in strains C57, D and A. In strain A a mature follicle was noted in 1 mouse. There was then a sharp decline in the occurrence of mature follicles in the transition from period

2 to period 3, and a still sharper decline in passing from period 3 to period 4; but there was no great difference in these respects between high and low mammary carcinoma strains.

Presence of Larger Than Normal or of Cystic and/or Hemorrhagic Follicles and the Phagocytic Activity of Granulosa Cells.—Hemorrhages in follicles are often associated with enlargement of follicles which is probably the primary change in such cases, although it is possible that in some instances a primary hemorrhage may have caused distention of a follicle. Hemorrhages are seen also in atretic follicles, where they may surround the egg. It is probable that in atretic follicles hemorrhages usually result when capillaries grow through the enlarged and perhaps vacuolated theca interna into the membrana granulosa, in the end reaching the central cavity. There may be a primary hyperemia which may be followed by hemorrhage. It is probable that in large follicles hemorrhages may be caused by hyperemia, such as occurs preceding ovulation. The enlargement of follicles and especially their cystic character are presumably due to a deficiency in the processes of maturation and ovulation, and such a deficiency may be caused by deficient function of the anterior lobe of the pituitary gland. Enlarged and cystic follicles often occur unaccompanied by hemorrhage, and, on the other hand, hemorrhage may occur in follicles of normal size. In 1 animal the clotted blood present in the cavities of several follicles had assumed a lamellated structure, and these blood clots were surrounded by phagocytic cells carrying blood pigment. In some instances it appeared that granulosa cells in follicles undergoing atresia were able to take up red blood corpuscles and also distintegrated cell material. Cells which were still part of the intact membrana granulosa were active rather than detached granulosa cells, although in the guinea pig the latter may also take up distintegrated cells during atresia of the follicles. These abnormal types of follicles may occur at all periods in the life of the mouse, but there are some differences as to the frequency with which they appear at various periods. All these types of follicles, but in particular very large follicles and occasionally even hemorrhagic follicles, may be found as early as period 1, but on the whole they are rare at this time of life. The majority appear in period 2 and still more in periods 3 and 4. Especially the cystic and cystic-hemorrhagic follicles are not usually seen in young mice. Likewise hemorrhages in atretic follicles are as a rule noted only from period 2 on, although they may appear even in period 1.

Occurrence of Primordial Follicles in the Ovaries at Various Periods of Life.—Primordial follicles or follicles approaching this state are not limited to period 1 but occur also in all the later periods. In period 4 they were seen in several strains, especially in the deeper parts of the

ovary between columns of interstitial gland cells and near the medulla. It is of interest to recall here the fact that I occasionally observed in the lumens of vessels, and likewise in the deeper portions, of the ovary of the guinea pig structures which apparently represented primordial follicles.

Atresia of Ovarian Follicles.—I have referred already to a characteristic difference of the follicular atresia in the mouse and the guinea pig. In the latter a massive disintegration of the granulosa cells takes place shortly after the onset of atresia, which is followed by phagocytosis of the disintegrated cells. In the mouse, on the other hand, the membrana granulosa may remain preserved for a relatively long time, but in the end connective tissue and blood capillaries may grow through the preserved granulosa and fill the cavity of the follicle. However, even in the mouse the onset of atresia does not as a rule leave the granulosa cells quite unchanged. They may undergo vacuolation with or without yellow pigment being deposited in the vacuolated cells. These atretic changes may occur during all periods of life. There may also take place, even during early stages of atresia, a solution of vacuolated granulosa cells, and this degeneration may occur in early as well as in later periods of life. In 1 case granulosa cells which were filled with small granules became transformed into colloid-like material. In hemorrhagic atretic follicles blood pigment may be the source of the yellow pigment seen in the granulosa. In strain C57 I observed that adjoining granulosa cells could unite into a syncytium and that the nuclei then could assume a rosette-like arrangement. It is significant that in strain C57 cells of the adrenal cortex situated near the medulla could join to form similar syncytia and rosettes. However, in a single instance the same change was observed in a follicle in strain C3H. As stated, hemorrhages were seen likewise in atretic follicles, and the hemorrhagic changes occurred especially in periods 3 and 4, therefore in older animals. But sometimes they occurred in period 2 as well. The hemorrhage under these conditions probably occurred when capillaries were growing into the granulosa and the coagulated blood could directly surround the egg. Under such conditions the vacuolar granulosa cells may take up red blood corpuscles or blood pigment. In rare cases granulosa cells were observed moving between the segments of segmented eggs in the centers of atretic follicles, and in 1 case a centrally situated egg was being destroyed by connective tissue growing through the granulosa. Connective tissue may begin to extend into the granulosa during early stages of atresia; gradually it reaches the inner aspect of this tissue and there forms a connective tissue membrane. If the inner layers of the granulosa have been destroyed, connective tissue may fill the cavity which has thus developed. With the connective tissue capillaries may penetrate into the granulosa and

hemorrhage may occur not only in the granulosa but in the theca interna of atretic follicles. Furthermore, during atresia the cells of the theca interna may enlarge and become vacuolar, and they may even carry yellow pigment, changes similar to those seen in the granulosa. In some instances it is possible to distingiush, in atretic follicles, theca cells from granulosa cells by the large size of the latter; moreover, as a rule, blood vessels are found in the theca. These changes in the theca cells may be noticeable as early as in period 1, but usually they develop only during periods 2, 3 and 4. The enlarged cuboidal theca cells can be seen at different stages to give origin to interstitial gland.

In some cases the enlargement of the granulosa cells and theca cells, together with the capillaries growing into the granulosa, and the lutein-like appearance of the latter, may lead to the formation of pseudo corpora lutea. This process differs from true ovulation in that rupture of the follicle and discharge of the egg do not take place. In some instances, however, it may be difficult to distinguish between an atretic follicle and a pseudo corpus luteum. In rare cases an atretic follicle may be transformed into a hyaline structure, and in 1 instance a hyaline layer was observed separating membrana granulosa and theca interna in an atretic follicle. Quite rarely nests of degenerated yellow granulosa cells can be recognized within the strands of interstitial gland tissue. In 2 mice which had been given injections of solutions of estrone and extracts of the anterior lobe of the pituitary gland for a considerable number of months the development of follicles ceased when they had reached small to medium size.

Life Cycle of the Corpora Lutea.—The corpora lutea result from the rupture of mature follicles. They grow, mature and then degenerate and gradually disappear. If stained with hemotoxylin and eosin during the process of their growth, they tend to take on a bluish tint; with increasing maturity this gives way to the red tint of eosin. At first the blood circulates well through corpora lutea; the capillaries are wide; at a later stage, however, when the decline of these bodies sets in, the capillaries become narrow and may contain polymorphonuclear leukocytes in large numbers. In this description of the life cycle of corpora lutea mention of their peculiarities during pregnancy has been omitted. In some cases it may be difficult to distinguish true corpora lutea from the pseudo corpora lutea which originate in atretic follicles through the enlargement of the granulosa cells, which, as stated, in certain instances become lutein-like; at the same time the theca cells may enlarge and become vacuolated. An egg is present in the center of such a structure. However, at least in the guinea pig it can be seen that in true corpora lutea, which have developed from follicles as a result of ovulation, the egg may occasionally be retained in the center of

a ruptured follicle. Thus in the mouse the presence or the absence of an egg may not furnish a decisive distinction between true and pseudo corpora lutea.

In some cases, furthermore, it is difficult to distinguish between a true corpus luteum and lutein-like interstitial gland tissue. This difficulty may arise in several conditions: (1) if the similarity between interstitial gland tissue and corpus luteum tissue is so great that only the sharp localization of the corpus luteum makes it possible to differentiate between these two formations, and this may not always be an easy task; (2) if the corpora lutea are pushed from the periphery of the ovary toward the center and here begin to degenerate, or if remnants of degenerated corpora lutea are included in the interstitial gland as one of its constituents; (3) if, during the degeneration of the corpora lutea, the latter are invaded and become partly replaced by interstitial gland; (4) if, as occurs under certain conditions, not only the true corpora lutea but also certain areas in the interstitial gland undergo a peculiar type of degeneration leading to hyalinization and gelatinization.

Mode of Degeneration of the Corpora Lutea.—Under ordinary conditions three processes take place during the degeneration of the corpora lutea: (1) vacuolation of the cells of the corpus luteum, which may begin in the central part of the corpus; (2) connective tissue invasion of the degenerating corpus luteum, followed (3) by shrinking of this structure. These processes constitute the usual degenerative change. At the same time the degenerating corpora lutea may be pushed from the peripheral parts of the ovary, the cortex, toward the center and the medulla, where they ultimately disappear; as stated, not only the connective tissue may invade perishing corpora lutea, but also the surrounding interstitial gland may do so, and in the end interstitial gland tissue takes the place of the disappearing corpus luteum tissue.

Hyalinization and Gelatinization of the Corpora Lutea.—There occurs in addition to the usual process just described a second type of degeneration consisting in hyalinization and gelatinization of these structures, leading at least in some cases to solution processes and occasionally also to deposition of calcium salts in the degenerated areas. This hyalinization can begin with deposition of a hyaline material in or around the walls of the arteries and of the capillaries within the corpora lutea; hyalin or a hyalin-like substance seems to develop also within the lutein cells. This mode of degeneration starts apparently in the peripheral parts of the corpus luteum and progresses toward the center. The stage of hyalinization is followed by a taking up of fluid by the hyaline tissue elements, leading to gelatinization, which may affect also the stroma of the corpora lutea. If this progresses, complete liquefaction of the corpus luteum and loss of nuclei, which prevent

distinguishing between corpus luteum tissue proper and stroma, take place. A precipitation of calcium salts may occasionally occur not only in the vessel wall, after parts of it have become necrotic, but also in the corpus luteum proper. In the end connective tissue may invade the hyaline-gelatinous corpora lutea from the periphery.

Various Other Hyaline Changes in the Ovary.—Often hyaline changes occur simultaneously in a number of corpora lutea of an ovary, so that a large portion of this organ as a whole becomes converted into hyaline or gelatinous material; finally the stroma elements which at first separate the various corpora lutea may disappear. Instead of steadily progressing general hyalinization-gelatinization, there were exceptionally noted some clumps of hyaline material in an otherwise not yet hyalinized corpus luteum, and foreign body giant cells could surround these hyaline clumps. But, in addition, more diffuse hyaline areas may occasionally be seen in ovaries, apparently unconnected with hyaline corpora lutea. Such hyaline areas may be present in various parts of the ovary, even at a distance from hyaline corpora lutea; they are especially prone to appear in older mice, and here hyaline blood vessels may extend into the tissue surrounding the ovary.

These arterial changes evidently correspond to the hyalinization of vessels which may appear in older individuals of other species. The question now arises (1) whether the hyalinization of an artery is the same process as the hyalinization of the corpora lutea, and (2) whether this process is a specific one of hyalinization or whether it represents an amyloid change. In the adrenal gland of the mouse my associates and I have attempted to distinguish between amyloid and hyaline changes by means of specific amyloid staining reactions, but without success. Also some other investigators did not succeed in obtaining the typical amyloid-staining reaction in the mouse. Under these conditions the question as to the chemical nature of this hyaline substance must be left undecided; but it is probable that the hyaline changes observed in the arteries of the corpora lutea and in other structures of the ovary and surrounding tissue are closely related to the hyaline changes which are found quite generally in blood vessels with advancing age. Processes of hyalinization and gelatinization have not been observed in the corpora lutea of the guinea pig.

Mode of Degeneration of Corpora Lutea in Various Strains of Mice.—When the various strains of mice were compared as to the mode of degeneration of the corpora lutea, no strain was found to be entirely free of the usual mode of degeneration, consisting in vacuolation, connective tissue ingrowth and shrinkage. Conversely, there was no strain in which hyaline or gelatinous changes failed to occur in corpora lutea, except perhaps strain CBA; but even in this strain it is not certain

that hyalinization might not have been observed if a still larger number of animals had been examined, especially in the later periods of life. On the other hand, the various strains differed greatly in the frequency with which these changes occurred in the corpora lutea and in the degree to which this mode of degeneration predominated over the usual one. Relative to the frequency and intensity with which hyalinization appeared, the various strains can be arranged approximately in the following order: CBA<C<C57 black<Old Buffalo< New Buffalo <C3H and AKA<D<A. In some cases the difference between adjoining strains was not definite. There is a strong indication that on the whole strains possessing a low incidence of mammary carcinoma have less tendency toward hyalinization than do the high mammary tumor strains. Thus the mammary tumor rate is low in strains CBA, C, C57 and Old Buffalo. In strain New Buffalo there is a medium mammary tumor rate. In strains C3H, D and A breeding mice the mammary tumor rate is high. However, while this relation between tumor rate and hyalinization is indicated, it is not absolute. Thus strain AKA, which possesses a very low mammary tumor rate, exhibits a strong tendency toward hyalinization and gelatinization of corpora lutea. Among the other strains there is no complete correspondence between mammary tumor rate and their tendency toward hyalinization and gelatinization. Strain CBA has less tendency toward hyalinization of corpora lutea than have strains C57 black and C, although the two last-named strains have a lower tumor rate than strain CBA. The results of this comparison again show that the use of only a small number of strains may lead to erroneous conclusions as to the correlation between certain strain characteristics.

Relation of Age to Incidence of Hyalinization.—In general the frequency of hyalinization of corpora lutea increases with age of the mice and this relation applies to strains in which hyalinization is a common occurrence as well as to those in which it is found relatively infrequently. However in those strains in which relatively few animals are affected, the occurrence of chance conditions may cover up the importance of age. Thus in strain C hyalinization occurred in period 1, although in this strain the change is on the whole rare. In period 3 an apparent transition of a normal to a gelatinous organ was noted, but there was no complete hyalinization in this case. As stated, in strain CBA no hyalinization was observed. In strain C57 there was in period 3 some hyalinization of corpora lutea as well as of arteries. Furthermore, in some other mice hyaline areas extended from the hilus upward toward the periphery. In period 4 no corpora lutea were recorded. In strain Old Buffalo 1 mouse was seen with hyaline corpora lutea in period 2. In period 3 of this strain there were some strands of hyaline tissue. In

period 4 a large gelatinous corpus luteum and a remnant of a gelatinous corpus luteum were noted. In strain New Buffalo about one third of the corpora lutea were hyaline or gelatinous in period 2. In period 4 large clumps of hyaline tissue were seen. In strain C3H some mice exhibited the usual mode of regression, while others had hyaline and gelatinous corpora lutea, in period 2. The greater part of the corpora lutea could be gelatinous; hyaline deposits occurred around vessels, and necrotic areas were found in hyaline vessel walls. In period 3 most corpora lutea were hyaline or gelatinous. In period 4 only 1 mouse was left for examination, and hyaline-gelatinous corpora lutea were seen in this animal. In strain AKA, in period 1 the usual mode of degeneration of corpora lutea predominated, but hyalinization did take place in some animals; also, transitional stages were noted. In period 2 the majority of the mice had corpora lutea in various stages of hyalinization, and in some animals almost all the corpora lutea were hyalinized. Also, there were hyaline arteries in association with hyaline corpora lutea. In period 3 in a large majority of the mice the corpora lutea were hyalinized, but some vacuolation still took place. Hyaline arteries were found in the ovarian tissue outside of corpora lutea. In period 4 there were shrunken remnants of corpora lutea. In gelatinous corpora lutea there were necrotic areas surrounded by foreign body giant cells. In strain D, in period 1 neither hyalinization nor gelatinization occurred. In period 2 the corpora lutea were mostly hyaline or gelatinous, but in rare instances degeneration by vacuolation took the place of hyalinization. In period 3 there were mostly hyaline or gelatinous corpora lutea, but there were also shrunken vacuolar corpora lutea with connective tissue ingrowth. In period 4 hyaline-gelatinous corpora lutea were found besides remnants of such corpora lutea. There was also hyalinization of vessel walls. Solution processes took place, and in other areas precipitation of calcium salts was present in gelatinous corpora lutea. In strain A, in period 1 degeneration of corpora lutea occurred mostly by vacuolation and connective tissue ingrowth, but in several older mice of this period hyaline or gelatinous corpora lutea were noted. In 1 mouse in which no hyaline corpora lutea were observed, a hyaline artery was seen, but hyalinization of arteries occurred also in hyaline corpora lutea. In period 2 a large majority of the corpora lutea were hyalinized or gelatinized, but in some ovaries vacuolar corpora lutea also were found, in addition to preserved corpora lutea. In an older mouse necrosis and calcium deposits were seen in the walls of an artery. There was also some vacuolation of corpora lutea. In period 4, likewise, a large majority of corpora lutea were hyaline or gelatinized; there were also isolated clumps of hyaline tissue, but even at this late time of life there were a few well preserved corpora lutea besides some remnants of vacuolar corpora lutea.

To summarize: In period 1 no hyalinization of corpora lutea was found in strains CBA, C57, Old Buffalo, New Buffalo, C3H and D. In strain C an exceptional case of hyalinization was observed. In strain AKA the usual mode of degeneration predominated, but hyalinization of corpora lutea occurred in some cases; also, transitional stages were seen. In strain A degeneration of the corpora lutea, as a rule, took place by vacuolation and connective tissue ingrowth, but in several older mice of this period hyaline or gelatinous corpora lutea were found. During period 1 there is, then, as a rule, absence of hyaline corpora lutea; only in strains AKA and A are mice affected by hyalinization or gelatinization of these structures, and those affected are but a small minority. Hyalinization and gelatinization of the corpora lutea therefore depend not only on the age of the corpora lutea but also on the age of the animals. In this sense hyalinization and gelatinization of corpora lutea may be considered as a phenomenon of aging.

In periods 2 and 3 the number of corpora lutea present was relatively great in the various strains and the animals had reached an older age. Accordingly the absolute number of hyalinized corpora lutea reached a maximum at this time. This was especially evident in strains in which the tendency toward hyalinization was strong, but even in the other strains the proportion of hyalinized and gelatinized corpora lutea was large. In strain C no typical hyalinization was found, but a corpus luteum showed a condition transitional to hyalinization in period 3. In strain CBA gelatinization was not observed. In strain C57 some hyalinization was noted in the corpus luteum in period 3. In strain Old Buffalo a single hyaline corpus luteum occurred in period 2. In period 3 some strands of hyaline tissue were seen. In strain New Buffalo about one third of the corpora lutea were hyalinized or gelatinized in period 2, while in period 3 hyaline-gelatinous corpora lutea were found in 2 mice. In strain C3H a minority of animals had hyaline or gelatinized corpora lutea in period 2, but in some of these animals the greater part of the ovary was gelatinized. In period 3 the majority of the corpora lutea were hyalinized or gelatinized and in some cases even the greater parts of the ovaries were gelatinized. In strain AKA, in the majority of the mice the corpora lutea were in various stages of hyalinization, and in some animals almost all the corpora lutea were hyalinized in period 2. In period 3, likewise, the majority of the corpora lutea were hyaline or gelatinous. In strain D much hyalinization and gelatinization of corpora lutea was seen during periods 2 and 3, and in strain A the large majority of the corpora lutea were hyalinized or gelatinized during periods 2 and 3. In strain New Buffalo there was a somewhat greater intensity of hyalinization than in strain Old Buffalo, and in strain AKA the hyalinization seemed to be more intense than that in strain C3H. Hyalinization was pronounced in strains D and A.

In period 4 degeneration of corpora lutea, presence of remnants of corpora lutea and in the end their complete destruction were noticeable. Well preserved corpora lutea were not frequent at this period. In strain CBA no corpora lutea were found in a number of mice; in others there was connective tissue ingrowth in degenerated corpora lutea, and remnants of corpora lutea were present; hyalinization was lacking. Only in 1 mouse were corpora lutea staining red with eosin seen. In strain C57 there were neither preserved corpora lutea nor the usual kind of vacuolated or hyaline-gelatinous corpora lutea, but in 1 animal two young vacuolar corpora lutea with some central hemorrhage were seen. In strain Old Buffalo there were large gelatinous corpora lutea and remnants of such corpora lutea, also vacuolar corpora lutea. In some mice leukemia caused lymphocytic invasion and destruction of corpora lutea. But there were 4 mice (2 were 28 months and 2 were 20 and 21 months of age) in which preserved corpora lutea were found. In some instances the distinction between true corpora lutea and corpus luteum–like interstitial gland tissue was difficult. In strain New Buffalo there were mostly degenerated vacuolar or hyaline remnants of corpora lutea, in addition to a corpus luteum that was invaded and split up by connective tissue. In strain C3H, in the single mouse left in this period, some good, as well as some hyaline-gelatinous, corpora lutea were found. In strain AKA no preserved corpora lutea were noted, but there were remnants of vacuolated and shrunken corpora lutea. There were also hyaline-gelatinous corpora lutea as well as remnants of such corpora lutea. In 1 breeding mouse a great part of the ovary consisted of gelatinous corpora lutea in which the nuclei had been lost. These necrotic areas were surrounded by foreign body giant cells. In strain D there were some preserved small copora lutea, but the larger corpora lutea were hyaline or gelatinous and in some places calcium deposits were found in gelatinous corpora lutea; also, solution processes, accompanied by loss of nuclei and followed by ingrowth of connective tissue, could occur in gelatinous corpora lutea. In 2 mice the corpus luteum tissue was tumor-like; it contained large nuclei and seemed to be slowly growing. In strain A the large majority of corpora lutea were hyaline and gelatinous. This process could advance so far that the greater part of the ovary became gelatinized and partly dissolved. A few small preserved corpora lutea were seen, however, in addition to some remnants of vacuolar corpora lutea. There were observed also clumps of hyaline material, which represented probably remnants of hyaline corpora lutea.

In these strains no strict parallelism between the tendency toward hyalinization of corpora lutea, the formation of new corpora lutea and the presence of preserved corpora lutea was seen. Thus in strain AKA, in which hyalinization was pronounced, preserved corpora lutea were

lacking in period 4. Furthermore, while there were some indications that the chances are greater that corpora lutea may be preserved at a later age of the mice in strains showing a high incidence of mammary carcinoma than in low mammary carcinoma strains, such a distinction between these two classes of strains is not definite. While in the high mammary cancer strains A, D and C3H preserved corpora lutea were found in period 4, some preserved corpora lutea occurred also in the low mammary cancer strains CBA and Old Buffalo during this period. Moreover in none of the strains studied during this time of life were there seen any new corpora lutea in contrast to the findings in period 3, where new corpora lutea appeared not infrequently in various strains, irrespective of whether these strains had a high or a low mammary cancer incidence or whether hyalinization of corpora lutea was a common or a more rare occurrence. In general in period 4 there was predominance of the various degenerative processes.

Growth Processes in Corpora Lutea.—In the follicles and corpora lutea abnormal, tumor-like growth processes were absent as a rule. However, as stated already, in strain D there were found in 2 mice in period 4 nuclear hypertrophy and increased size of corpora lutea. In former investigations occasional mitoses were noted in corpus luteum cells of the guinea pig. There have now been found mitotic figures in corpora lutea in period 1 in three strains of mice; in strain D they were observed in well preserved adult corpora lutea, and in strain A a mitotic figure was seen in a young corpus luteum.

SUMMARY

A decline of the full development of follicles sets in in period 3 and becomes more pronounced in period 4. However, differences are observed in this respect between different strains; there is no parallelism between the fate of the follicles in different strains and the tendency of these strains to have mammary carcinoma. A sharp decline in the number of mature follicles takes place during period 3 and still more during period 4. While in this respect, also, a difference is observed in different strains of mice, it does not correspond to the division of mice into high and low mammary carcinoma strains.

Follicles may be very large, and cystic or hemorrhagic, or cystic and hemorrhagic at the same time. It seems that in some cases dilatation of the follicles is the primary process, while in other cases hemorrhage is primary. Hemorrhages may also be found in atretic follicles. Under certain conditions the granulosa cells may act as phagocytes toward constitutents of the extravasated blood or they may take up disintegrated cell material.

In mice primordial follicles may be seen in the deeper parts of the ovary, even in older animals; likewise in the ovary of the guinea pig

they have been observed in the blood vessels in the deeper parts of the ovary.

The atresia of follicles of mice differs in some respects from the corresponding process in guinea pigs. A number of changes are described which may be observed in atretic follicles in mice.

The corpora lutea of mice undergo a definite life cycle, but in some cases it may be difficult to distinguish between corpora lutea and pseudo corpora lutea or between true corpora lutea and certain types of interstitial gland.

Besides the usual type of degeneration of the corpora lutea, in which essentially three processes take part, there is a second type consisting in processes of hyalinization, gelatinization and solution. The hyalinization of the corpora lutea may begin in or around their blood vessels, and the problem arises as to the nature of the hyalinization occurring in the blood vessels and in the corpora lutea proper; in the latter hyalinization is perhaps related to amyloid changes, which in the mouse cannot—at least not in all cases—be clearly distinguished from certain other processes by specific staining reactions.

Both types of degeneration of corpora lutea were encountered in all the strains examined except perhaps strain CBA, in which so far neither hyalinization nor gelatinization has been observed. However, the proportions in which these two types, represented by vacuolation and hyalinization, occur differ greatly in different strains. On the whole hyalinization and gelatinization predominate in strains possessing a high mammary tumor rate; but this difference between high and low mammary tumor strains is not absolute. Strains AKA, which has a very low mammary tumor rate, has a strong tendency toward hyalinization of the corpora lutea, and strain CBA, which has a higher tumor rate than strain C57 black, has less tendency to hyalinization of the corpora lutea than strain C57. A consideration of only a small number of strains would therefore lead to erroneous conclusions as to the correlation between the frequency of occurrence of certain characteristics and some other properties of these strains.

The hyalinization and gelatinization of corpora lutea increase in frequency and intensity with increasing age of the mice up to period 4. In the last period of life destructive processes predominate in corpora lutea. There was no complete correspondence between the greater or the lesser tendency of corpora lutea to undergo hyalinization and the presence of preserved corpora lutea in the various strains of mice.

Tumor-like formations of corpora lutea were found in only 2 mice of strain D.

Mitotic figures can be seen in corpora lutea of mice as well as in those of guinea pigs, although they are rare in both species.

II. THE FATE OF THE INTERSTITIAL GLAND AND THE FORMATION OF OVARIAN DUCTS AND CYSTS

IN THE first part of these investigations the fate of follicles and corpora lutea was described, and it was shown that with advancing life of the mice follicles and corpora lutea disappear more and more and their place is taken by the interstitial gland and various duct formations. In this part, the changes taking place in the latter two substitutional structures will be followed from the first to the fourth period of life. I shall proceed by describing more fully the alterations which occur in these two formations in one low mammary cancer strain, CBA, and in one high mammary cancer strain, D, and I shall then compare the development of the interstitial gland and the ducts in these two strains with their evolutions in the other strains.

OBSERVATIONS IN STRAINS CBA AND D

STRAIN CBA.—*Period 1.*—As usual, during period 1 the interstitial gland consisted of small and medium-sized, more or less cuboidal cells. Groups of typical yellow-pigmented interstitial gland cells were mostly lacking, but some yellow degenerated granulosa cells were seen in atretic follicles, which were found occasionally among the groups of interstitial cells.

Ducts derived either from germinal epithelium or from medullary tissue were not conspicuous.

Period 2.—The interstitial gland was similar to that seen in period 1, but in 5 of 14 mice yellow cell nests developed in the interstitial gland. In 1 mouse there were merely some yellowish brown cells, which seemed to be remnants of the membrana granulosa of atretic follicles; also in another mouse granulosa of atretic follicles seemed to be present in the interstitial gland. In still another animal there were in various places of the interstitial gland packages of yellow and pinkish vacuolar cells, which may have been derived from old corpora lutea. The yellow cells seen in this strain were smaller and less numerous than those in strain C57 black, and the vacuolar yellow cells were larger than the ordinary interstitial gland cells. These yellow cells, also, may have been derived at least in part from the granulosa of atretic follicles.

In 3 mice medullary cysts were found; but in only 1 animal were ducts seen in the cortex.

Period 3.—In the older group of mice, 17 to 20 months old, the ovaries consisted mostly of interstitial gland tissue. This was in part composed of small cuboidal cells with vesicular nuclei. These cells became larger in the cortex, and there were here more groups of yellow vacuolar cells admixed to these cells, especially near the subgerminal spindle cell tissue. Some of these yellow cell nests may have been derived from atretic follicles. In general there were large amounts of yellow interstitial gland tissue in these ovaries. Also in the younger mice, 15 to 17 months of age, the interstitial gland tissue represented the largest part of the ovary. The amount of yellow vacuolar cells was perhaps somewhat smaller than

in the older mice, but there was great variation in this respect. In the cortex there was a small number of groups of reddish and small, pale yellow cells; they may perhaps have been remnants of corpus luteum tissue. But most of the interstitial gland consisted of small or medium-sized cells or of somewhat larger cells: the last appeared almost lutein-like. Different degrees of vacuolation were found, and cellular fibrillar connective tissue strands separated various areas in the lutein-like tissue. In the center of the ovary dense hyaline material was found; it may have been derived from atretic follicles. In some places there were groups of cells which resembled large granulosa cells in transition to corpus luteum tissue.

Several medullary ducts were found. Cortical ducts accompanied by subgerminal spindle cell tissue could be seen to move downward into the deeper ovarian areas; these ducts could be lined with cuboidal epithelium. Medullary ducts surrounded by interstitial gland tissue extended upward toward or into the cortex. These ducts were lined with cylindric epithelium or with yellow vacuolar cells; the higher up they penetrated the smaller they became. The layer of subgerminal spindle cells sent processes downward; they surrounded cell nests, possibly remnants of some atretic follicles.

Period 4.—Interstitial gland structures were here so intimately mixed with duct formations that these two constituents could not always be sharply separated. In the older group of mice, ranging in age between 26 and 31 months, there were adenomatous formations situated in groups of interstitial gland cells and covered by germinal epithelium; these formations protruded into a germinal cyst overlying the ovary. It seemed that some ducts, lined with cuboidal vacuolar cells and surrounded by subgerminal spindle cell tissue, gave origin to these adenomatous structures. Papillae filled with yellow interstitial gland structures protruded in places into these germinal ducts. Some ducts were lined with flat epithelium, and in others there were pearls consisting of squamous epithelium, which could be partly hyaline. Mitotic figures were seen in the epithelial lining of the ducts. In some places the duct epithelium became vacuolated and yellow. It seemed that groups of yellow cells situated in the ovarian stroma were derived from such ducts filled with vacuolar yellow cells. Some of these vacuolar cells could coalesce and form syncytia. In other places interstitial gland consisted of small cuboidal cells possessing small nuclei; these cells were probably derived from the theca interna of atretic follicles. Groups of somewhat larger cells may possibly have represented remnants of corpus luteum tissue. The groups of yellow cells were in places so large that they exerted pressure on the connective tissue stroma and destroyed it. But also medullary ducts were in intimate connection with interstitial gland, and it was difficult to determine whether they were merely in close contact with it or whether they changed into this tissue and thus gave origin to it. Underneath the germinal epithelium there could be found either the usual layer of spindle cells or a layer of hyaline material. The spindle cell tissue often penetrated deep into the ovary, either around ducts or between strands of interstitial gland, forming partitions between the latter. Deeper toward the center of the ovary connective tissue fibers gradually developed between the spindle cells. Likewise hyaline strands penetrated into the interstitail gland and separated it into bundles of interstitial gland cells. In the younger group of animals conditions on the whole were similar to those observed in the older group, but in the younger animals the structure of the ovary was perhaps a simpler one,

inasmuch as groups of smaller or medium-sized cuboidal cells became more prominent. These formations resembled those seen in earlier age periods. However, in these animals also vacuolation and formation of yellow pigment occurred. It seemed that the yellow cells could in some instances contain blood pigment. Likewise medullary ducts lined with vacuolar cells seemed to give origin to interstitial gland.

As to ducts and cysts, in the older mice one occasionally found germinal epithelial cysts and also medullary cysts. Yellow vacuolar cells lined some of the ducts, and large vacuolar cells were seen in the lumens of the tubules. There were also coiled ducts containing yellow cells, and mitotic figures were seen in ducts. Pearl-like bodies which centrally contained keratin or concentric lamellar hyaline material were found in large, cystically dilated ducts and were derived from their lining cells. Strands of subgerminal spindle cells could accompany the ducts, and some of the latter penetrated into the fat tissue surrounding the ovary. Ducts lined with high cylindric epithelium or, in other cases, with vacuolar light-colored or yellow cells originated probably from medullary ducts. They penetrated upward into the interstitial gland and seemed to destroy it in places. Mitotic figures occurred in the epithelium of the medullary ducts. A great part of the ovary consisted of small ducts lined with low cuboidal epithelium; they were surrounded by subgerminal spindle cell tissue. These ducts were probably derived from germinal epithelium. They grew downward into the center of the ovary, while medullary ducts could penetrate upward, even as high as into the subgerminal connective tissue. Some of the germinal ducts could be coiled, and they could be surrounded by dense hyaline strands which took the place of the spindle cells. Both medullary and germinal ducts seemed to change in various places into interstitial gland cell tissue, and thus they were apparently able to produce interstitial gland tissue, including yellow cell strands. Medullary ducts seemed to form also lutein-like tissue or to penetrate into packages of yellow cells.

Strain D.—*Period 1.*—The interstitial gland cells were mostly small or medium sized, although their size varied to some extent in different places; in some areas they were granular or filled with small vacuoles. In mice 3 or 4 weeks old the interstitial gland was as yet rudimentary or lacking altogether. Yellow interstitial gland cells were in evidence only in 1 nonbreeding mouse, not quite 8 months of age and weighing only 17 Gm., and in 2 breeding mice. There seemed to exist an inverse relation between the size of the follicles and the amount of interstitial gland that had developed. In 2 animals the vessels of the medulla were much enlarged.

Ductlike processes given off by the germinal epithelium and penetrating deeper into the ovarian tissue were not recorded; but in 1 mouse a large medullary cyst was observed.

Period 2.—The interstitial gland resembled that seen during period 1. In a number of mice there were groups of larger, usually vacuolated yellow cells, the pigment being derived in some instances from blood pigment taken up by these cells, which were thus acting as phagocytes. Such yellow cells were found in the medulla, especially around the vessels. But also some regressing corpora lutea seemed to have been incorporated in the interstitial gland tissue.

Ducts, in contrast to the first period, in which they were absent or rare, were more frequent in period 2. Among the older mice they were prominent in 5 animals. In one of these there were in the cortex convoluted ducts, which were probably derived from the germinal epithelium. In a second one there were, in addition to invaginations of the germinal epithelium, medullary ducts with papillae and a large papillary cyst of medullary origin. In a third mouse there was a duct, probably of medullary origin, lined with cells resembling sebaceous

gland cells. Likewise in another animal there was a medullary cyst, and in the last mouse most of the ovary was enclosed in a cyst, which presumably was derived from the germinal epithelium. In the center there was a conglomerate duct. In the second oldest group of mice, there was in 1 animal, which had received an injection of potassium iodide, underneath the germinal epithelium, a collection of large ducts of germinal epithelial origin. There was also a conglomerate duct in the center, lined with cylindric epithelium; it may perhaps have been the continuation of a medullary duct. In addition there was in the medulla a large body consisting of large polyhedral cells not unlike liver cells. In a second mouse of this group there were some coiled ducts in the center of the ovary and in a third mouse a medullary cyst was seen. In the third group medullary cysts were noted in 2 mice; in one of these there was, in addition, in the hilus an angioma-like network of widely dilated capillaries which reached to the capsule of the cortex. Pressure exerted by the blood vessels destroyed the tissue situated between the dilated capillaries. In a third mouse of this group dilated lymph vessels were found in the medulla. In still another group of mice large cysts, probably of medullary origin, were found in 3 animals. In 1 breeding mouse invaginations of the germinal epithelium were seen. In still another animal an adenoma-like structure was present; it was derived from medullary ducts. In 2 other mice cysts were noted, lined with cylindric epithelium and surrounded by interstitial gland.

Period 3.—The interstitial gland consisted mainly of small or medium-sized cells derived probably from the theca interna. There was also much lutein-like tissue, which may have originated either in hemorrhagic corpora lutea or in interstitial gland that had changed into lutein-like tissue. Great numbers of largely hyalinized or gelatinized corpora lutea, which represented the greater portion of the ovaries in a number of mice, exerted pressure on the interstitial gland, which was thus reduced in size. Yellow interstitial gland tissue was not prominent. Hyaline material infiltrated the interstitial gland in places, while in other places there was extreme hyperemia in remnants of this tissue. In one ovary ducts seemed to give origin to interstitial gland.

Ducts originating in the germinal epithelium were numerous; there were also medullary ducts, and the latter could become converted into medullary cysts. Some ducts could connect with interstitial gland tissue, in particular also with lutein-like tissue, and in such cases again it appeared as if they gave origin to this tissue. Subgerminal spindle cell tissue accompanied ducts into the deeper parts of the ovary, and some of these ducts consisted of several layers of cells, which at first were solid but gradually seemed to develop into larger vacuolated or yellow cells. There were mitotic figures in some of the cells of the duct epithelium. In other places ductlike structures connected with tissue which was composed of large cells with large vesicular nuclei; the cytoplasm of some of these cells could be reduced in amount, and in this tissue mitotic figures also could be present. In 2 mice, 19 months old, adenoma-like structures developed; they seemed to have arisen from prolongations of the germinal epithelium, and they appeared to be composed, at least in some places, of conglomerated ducts. In one of the 2 mice these formations were present in both ovaries, and they reached into the interstices between gelatinized corpora lutea; they were covered by germinal epithelium and contained some large vessels.

Period 4.—In a 22 month old mouse almost all the ovary had become gelatinized, and the gelatinized area included apparently also the interstitial gland; there was only a small amount of typical interstitial gland left, and it contained a few vacuolar yellow cells. This observation suggests that hyalinized corpora lutea may be able to contribute to the formation of the interstitial gland. There was also

present a tumor-like structure consisting of coiled ducts containing mitotic figures; it was probably derived either from the germinal epithelium or from medullary ducts. A considerable part of the ovary was replaced by dilated capillaries, between which only some remnants of ovarian tissue were left. In a 21 month old mouse there was a corpus luteum–like structure which seemed to have given origin to interstitial gland. An artery in the tissue surrounding the ovary was ensheathed by dense hyaline tissue. In a 20 month old animal ductlike structures appeared to change to interstitial gland. There were nests of cells with vesicular nuclei, each of which contained a single central neucleolus. Most of the ovary consisted of interstitial glandlike tissue which seemed to have developed from corpora lutea, and a great part of this interstitial gland tissue was situated around vessels. In addition there were structures consisting of coiled ducts which could resemble corpus luteum tissue. In the breeding mouse small-celled interstitial gland tissue was seen.

Ducts penetrated from the germinal epithelium into the deeper ovarian tissue, and subgerminal spindle cell tissue reached a little into gelatinous lutein tissue. In 2 animals ducts gave rise to tumor-like structures, in one of which mitotic figures were present. In some instances it was difficult to decide whether ducts were of germinal epithelial or of medullary origin; this was particularly so in the case of the coiled tumor-like formations mentioned. As stated, some of these ductlike structures seemed to change into interstitial gland tissue. In the breeding mouse a large papillary cyst was seen; it was lined by cylindric epithelial cells. Blood vessels were present in the papillae which protruded into the cavity of the cyst, and the latter contained a colloid material. In this case, also, it was uncertain whether the cyst was of germinal epithelial or of medullary origin, and in this case also, the beginning of an excess formation of ducts was noted.

COMPARISON OF CHANGES IN INTERSTITIAL GLAND AND DUCTS TAKING PLACE WITH INCREASING AGE IN STRAINS C, AKA, OLD BUFFALO, NEW BUFFALO, C57 BLACK, C3H AND A WITH THOSE NOTED IN STRAINS CBA AND D

I shall describe more briefly how far conditions found in various other strains agree with those described in strains CBA and D.

Strain C.—Only mice which were in periods 1, 2 and 3 of life were available; mice of period 4 were lacking. In the interstitial gland conditions were about the same as in that of strain CBA. In the main this tissue seemed to be derived from the theca interna of atretic follicles, but a participation of corpora lutea could not be excluded in every case.

Ducts were not conspicuous in period 1, and they were on the whole infrequent in most animals of this strain during periods 2 and 3.

Strain AKA.—In period 1 the interstitial gland was typical. In some instances it was arranged in strands between large capillaries in the medulla of the ovaries. With increasing age the size of the interstitial gland, as well as the number of yellow or brownish pigment cells, increased. In period 4 groups of yellow, often vacuolated cells were prominent, and some of the pigment seemed to have been derived from the hemoglobin of red blood corpuscles. In some packages of yellow cells the nuclei were lost. Ducts of germinal epithelial origin, as usual, were not noted in period 1; they began to become prominent only in period 2. In period 3 medullary ducts, also, were numerous. A medullary cyst exerted pressure on the ovary proper. In period 4, also, enlarged medullary ducts were seen, and some of the ducts in the deeper region of the cortex were filled with vacuolar

yellow cells; these ducts seemed to give origin to interstitial gland. There were also collections of ductlike structures, with or without lumens, lined with rather large, well developed cells, some of which had two nuclei. There were some mitotic figures in these collections of ducts, which resembled adenoma. It was difficult to decide whether these ductlike structures had actually been converted into interstitial gland. In the medulla there were some arteries whose muscularis was hyaline; there was also a thrombosed artery in process of fibrosis.

Strain Old Buffalo.—In principle the conditions were similar to those observed in the other strains; old age changes became prominent only in period 4. There was, however, a difference of interstitial gland in Old Buffalo and in other mice in so far as this tissue was larger in amount in the Old Buffalo mice and that there was here much yellow interstitial gland tissue. Also the germinal epithelial ducts as well as the medullary ducts could become very numerous. The cells lining the ducts in places enlarged, became vacuolar and yellow and connected with interstitial gland tissue consisting of lutein-like cells. Such conditions suggested strongly that ducts were transformed into interstitial gland tissue. Ducts could also become coiled or convoluted and give rise to complex structures which likewise seemed to change into interstitial gland. In the hilus of the ovary were collections of alveolar formations which consisted of cells rich in cytoplasm. Corpora lutea, in which there was some indication of hyalinization, as well as atretic follicles with remaining granulosa cells, seemed to become incorporated in places in the interstitial gland. The subgerminal spindle cell tissue often accompanied germinal epithelial ducts downward into the ovary; but these strands of spindle cell tissue could penetrate still farther into the ovary in places, and they surrounded groups of yellow or light vacuolar interstitial gland cells. Instead of spindle cell tissue, strands consisting of hyaline material penetrated into the ovary in other places; they separated groups of interstitial cells and could also surround capillaries and larger vessels. Very characteristic was the invasion of the fat tissue surrounding the ovary by ducts with accompanying lutein-like interstitial gland and also by coiled duct formations. This indicated that these structures had assumed tumor-like growth tendencies. Cysts on the whole were not common in this strain, but in period 4 some papillary cysts of germinal epithelial origin were found. In these papillae there were blood vessels surrounded by groups of yellow vacuolar cells with vesicular nuclei. As in other cases, here also the problem arose as to whether these ducts themselves gave origin to the lutein-like tissue and to the yellow interstitial gland tissue, or whether they merely invaded the preformed lutein-like tissue. All these changes, on the whole, were more prominent in the older than in the younger mice of period 4.

New Buffalo Strain.—The interstitial gland showed the typical age changes. In periods 1 and 2 the interstitial gland had the character of a young tissue, although the size of some interstitial gland cells and the number of yellowish brown cells it contained were greater in period 2 than in period 1. In period 3 a notable change in the condition of the interstitial gland occurred. Its relative size increased, as well as the size of many constituent interstitial gland cells, which first became vacuolar and then acquired yellowish brown pigment. Blood pigment seemed to be contained in some of these cells, and this was in accord with the fact that hemorrhagic areas were seen in the cortex or the medulla of the ovary and that there were in places dilated blood vessels, which could give rise to hemorrhages, as well as lymph vessels filled with lymphocytes. Also some hyaline arteries or hyaline areas appeared at that time. In period 4 these changes progressed. Hyalinization of the intima and media of arteries became more frequent, and there

were occasionally also hyaline rings around capillaries. The development of yellowish brown cell areas likewise progressed, and they were seen in the center of groups of nonpigmented vacuolar cells, from which presumably they had taken their origin.

Ducts, on the whole, were scarce in periods 1 and 2, although they were somewhat more frequent in period 2. As in the interstitial gland, so also in the ducts a noticeable increase took place in the number of both types of ducts in period 3. Also, the layer of subgerminal spindle cells was well developed in the cortex. It resembled distinctly the corresponding tissue found in the cortex of the adrenal gland. In both adrenal gland and ovary, from the peripheral areas of the cortex the spindle cell tissue may grow downward into the deeper parts of these organs, and in the ovary it may reach between the strands of the interstitial gland, although such a downgrowth was rare in period 3. In certain instances the slit which is lined by germinal epithelium on top of the ovary enlarged into a cyst. In some animals, not in all, papillae protruded into such a cyst, and ducts developing from the germinal epithelium reached down into the ovary. In several cases not only medullary ducts were found, but also medullary cysts which developed from the ducts. There was thus an increase in the cyst formation in period 3. In this period there were pictures suggesting a transition of some cortical as well as of medullary ducts leading to small cell or larger cell, vacuolar or pigmented interstitial gland tissue.

In period 4, in the oldest mice, between 2 and 3 years of age, the number of cortical ducts could be much reduced, while in medulla and hilus there were large convoluted ducts, the lumens of which could be filled with epithelial cells. In younger mice of this period some germinal ducts formed alveoli which were filled with solid cells. Some medullary ducts were surrounded by hyaline tissue, and directly underneath the cortical germinal epithelium a hyaline membrane could be seen covering a layer of spindle cell connective tissue cells. In this strain the development of the cortical ducts was therefore deficient in the oldest mice, but there was again an increased formation of hyaline structures.

Strain C57 Black.—C57 black differs from other strains in these respects: The interstitial gland differentiates more rapidly. As early as in period 1 yellowish brown tissue is relatively strongly developed, and there is already noticeable in this tissue the tendency to form syncytia; as a result the cell membranes between adjoining cells which are involved in this process disappear. In the course of time it was found that in these syncytia the nuclei could arrange themselves in a radiating direction, so that rosette-like structures developed. In contrast to the interstitial gland, the production of ducts remains relatively rudimentary in these mice, and the changes of ducts into adenoma-like structures and apparently also into interstitial gland are not so pronounced as in the other strains, although in period 4 the formation of ducts and the secondary changes which may take place in the latter are pronounced. It is of interest that in the adrenal gland of strain C57 black the development of yellowish brown pigmented cells with rosette-like arrangement of the nuclei was especially pronounced. It seems therefore that this structural peculiarity is not altogether due to conditions confined to the ovaries but is to be ascribed to more generalized conditions which are active also in other organs.

It is perhaps owing to the strong development of the interstitial gland that the ducts do not find a chance to grow out in large numbers toward the center of the ovary. In the various periods one may note in addition the following findings: In period 1 interstitial gland tissue was present as early as 4 or 5 weeks of age, although it was as yet smaller in amount at this age. In mice younger than 6 weeks yellow cell strands were lacking, and in mice up to 3 months of age they

were smaller in size. As in the other strains, so also in C57 black the pigment in some of these cells seemed to be derived from hemoglobin, and this applies also to a number of the animals in the later periods of life. Some germinal epithelial ducts were present in a breeding mouse, and in the lumens of other ducts there were phagocytic cells containing blood pigment or erythrocytes. In period 1 as well as in period 2 the interstitial gland seemed to take its origin largely from the theca interna of atretic follicles, and only occasionally regressing corpora lutea appeared to take part in its formation. In some places the large interstitial gland cells developed perhaps in part from the membrana granulosa of atretic follicles, which under certain conditions seemed to give origin also to rosette formation.

In period 2 the large yellow cells or the syncytia were preferably situated around blood vessels, especially in the medulla. Hyaline material in large amounts could surround vessels entering the ovary. This hyaline substance was probably different from the hyaline that develops in great amounts in the corpora lutea in various strains but only to a slight extent in strain C57 black. In some places large interstitial gland cells seemed perhaps to develop from the granulosa of atretic follicles, which, as stated, may be able under certain conditions to give origin to rosette formations.

In period 3 it appeared that the groups of yellowish brown cells could in places develop from the smaller cuboidal cells. The differentiation of interstitial gland was especially pronounced in the medulla of the ovary where this tissue was oldest. Yellow cells were frequently situated around large blood or lymph vessels in the medulla. Ducts of medullary and germinal epithelial origin were more frequent in period 3, as well as cysts derived from medullary or from germinal epithelial ducts.

In period 4 the interstitial gland resembled the formations observed in the preceding periods, but in addition there was an indication that a part of the interstitial gland developed from ducts growing in the ovarian tissue. In some places strands of yellow interstitial gland cells were observed breaking through the ovary into the surrounding fat tissue. In the medulla hyaline vessels were seen. Germinal epithelial ducts were somewhat more frequent in this period and they likewise could penetrate into the fat tissue surrounding the ovary. In 1 mouse there was present in the fimbria an adenoma-like structure which seemed to have taken its origin either from the germ epithelium or from the epithelium of the fallopian tubes. Some of the cells of this structure were vacuolated. There occurred also in the ovary convolutions of ducts lined with vacuolar epithelium which in 1 case appeared to give origin to interstitial gland. Near the hilus of the ovary there were some medullary ducts lined with high epithelium or with several layers of epithelium. Underneath the germinal epithelium typical subgerminal spindle cell tissue was noted in some places, while in other places a layer of fibrillar connective tissue was found. Dilated blood capillaries in the outer region of the cortex could give origin to hemorrhages, and strands of subgerminal tissue penetrated into the coagulum. In this strain, as also in some other strains, masses of lymphocytes could destroy various parts of the ovary in some mice—an indication that a certain number of animals were probably affected by leukemia.

Strain C3H.—At the earlier periods the interstitial gland tissue consisted predominantly of small or medium-sized cells which seemed to develop especially around atretic follicles and to originate from the theca interna of these follicles; the latter, accordingly, could perhaps be included in places in the interstitial gland. It is not probable that remnants of corpora lutea took a significant part in the formation of interstitial gland tissue, inasmuch as they often undergo hyalinization in this strain and such hyalinized areas were not visible in the interstitial gland. How-

ever, hyaline vessel walls were seen in period 4. The formation of yellow interstitial gland tissue remained on the whole moderate, and phagocytosis of erythrocytes could here also contribute to the production of yellow pigment. The interstitial gland was especially prominent in the medulla where dilated lymph vessels could traverse it. Ducts were moderate in number in periods 2 and 3, but in period 4 large amounts of convoluted ducts could give rise to adenomatous areas consisting apparently of masses of alveoli. These alveoli were surrounded by subgerminal spindle cell tissue—an indication that these formations were derived from the germinal epithelium which gave origin to ducts. In places pressure exerted on the subgerminal strands of spindle cells by the underlying tissue caused the destruction of the spindle cell layer.

Strain A.—In periods 1 and 2 the interstitial gland showed the structure characteristic of young animals. Yellow tissue was seen only rarely, although more often in period 2 than in period 1. Areas of hyalinization which occurred in period 2 in the interstitial gland may perhaps have been due to admixture of hyaline corpora lutea. Even in period 1 the possibility that corpora lutea participated in the formation of the interstitial gland could not be entirely excluded. In the youngest mouse, 6 weeks of age, no typical interstitial gland was seen. In period 1 also a large hemangioma-like body was found at one end of the ovary. In another mouse there were dilated lymph vessels in the medulla. Only a small number of ducts, mainly of medullary origin, but exceptionally also derived from the germinal epithelium, were seen.

In period 2 a large medullary cyst appeared. In 1 case a ductlike structure seemed to give origin to lutein-like tissue; but usually such formations were seen only in later periods.

In period 3 the vacuolation and formation of large vacuolated yellow cell groups made some progress. Some hyalinization was observed in the interstitial gland as an indication that hyalinized corpora lutea may have contributed to its formation. In the membrana granulosa of atretic follicles vacuolation and advancing degenerative changes could be observed. In the hilus of the ovary hyalinized arteries were seen. At this period germinal epithelial ducts were on the whole more frequent, although they were not prominent in all the mice. The slits lined with germinal epithelium and covering the ovaries in some cases could become transformed into cysts. Also medullary ducts lined with cylindric cells and containing cytoplasmic granules were observed.

In period 4 the interstitial gland underwent changes similar to those observed in other strains in older mice. Areas with large, yellow, vacuolated lutein-like cells, lining ducts, increased in size. These cells could exert phagocytic activity toward erythrocytes. In places dilated blood vessels gave rise to hemorrhages enclosed in cystlike formations in the interstitial gland tissue. Mitotic figures could occur in the vacuolar interstitial gland cells. The media and the intima of some vessels were hyalinized. The layer surrounding the arterial endothelium was infiltrated in various places with red corpuscles, so that these areas assumed a red stain with eosin. Also arteries seen in the tissue surrounding the ovary could be strongly hyalinized. Hyalinized and gelatinized areas in the interstitial gland suggested the inclusion of hyalinized corpora lutea in this tissue, but in addition very large follicles could be seen in which the outside cells, apparently corresponding to theca interna cells, were vacuolar and the inside cells, representing presumably granulosa cells, were lutein-like. It appears probable that the interstitial gland was derived at least partly from theca interna cells which had undergone corresponding changes. Germinal epithelial and medullary ducts, some of which were convoluted, could be lined with ordinary vacuolar cells or with

yellow vacuolar cells, and such cells could also fill the lumens of the ducts. Mitotic figures were seen in the cells of these ducts. It appeared as though these ducts gave origin to the lutein-like interstitial gland tissue. In several mice the slits lined with germinal epithelium enlarged into cysts which could be filled with blood. These hemorrhages resulted from the presence of much dilated vessels near the cysts. These cysts could be surrounded by subgerminal spindle cell tissue in accordance with their origin from germinal epithelium. One sees, then, in strain A changes in the interstitial gland which in principle are similar to those seen in other strains.

COMMENT

I shall now compare the variations which occur (1) in the interstitial gland structures and (2) in the ducts in the various strains of mice with increasing age.

Origin of the Interstitial Gland and Variations in the Amount of Interstitial Gland Tissue.—In general, and this applies with some reservations to all the strains, the interstitial gland increases in size with increasing age of the mice. In very young animals, 3 to 5 or 6 weeks old, the interstitial gland was as yet very rudimentary or lacking almost entirely. However, in strain C57 black in which this tissue shows a more rapid development and differentiation and more pronounced changes with old age than in some other strains, interstitial gland was already present in mice 4 to 5 weeks old. In periods 1 and 2 it consisted mainly of small and medium-sized cuboidal cells, but it was found in somewhat larger quantity in period 2 than in period 1. It underwent more marked changes in period 3 and especially in period 4. At this time it usually increased in size; the individual cells enlarged, and they tended to show more or less vacuolation and to accumulate yellow pigment. The largest quantity of the interstitial gland was often found in the medulla, where it inclined to be arranged especially around the larger vessels. As to its origin, it seems to be derived, at least to a considerable extent, from the theca interna of atretic follicles; but there are indications that corpora lutea, also, and even the membrana granulosa of atretic follicles may perhaps take part in its formation, as I shall discuss later. Variations, besides those already mentioned as occurring in strain C57, may depend on the prominence of the corpora lutea. Thus, especially in strain D, during periods 3 and 4, hyalinized and gelatinous corpora lutea were prominent. They could press on the interstitial gland, which was thereby diminished in amount. On the other hand, if the interstitial gland was strongly developed, it also could press on adjoining tissues and cause their destruction. Thus in strain C3H it was observed that pressure exerted by the interstitial gland on the subgerminal spindle cell tissue led in places to the destruction of the latter.

Origin and Relative Quantity of the Vacuolar and Yellow Interstitial Gland Tissue.—In general, yellow cells, which usually are vacuolar and

larger than the young cuboidal interstitial gland cells, are present only in small quantities in the interstitial gland in period 1 or they may be absent at that time. They increase somewhat in period 2. In strain CBA yellow pigment seemed to appear also in the membrana granulosa of atretic follicles. In strain D, in period 2 the yellow interstitial gland cells were situated especially around vessels in the medulla. In strain C57 there was yellow interstitial gland tissue present in period 1, and syncytia formed in this tissue as a result of the coalescence of neighboring cells. The nuclei could be arranged in the form of rosettes. In mice younger than 6 weeks yellow cell strands could already be seen, but from this time on up to the age of 3 months they were smaller in size. Also in the adrenal gland of strain C57 yellow cells, syncytia and rosettes were present. In regard to the yellow tissue we have therefore to deal with a peculiarity which is not limited to the ovaries. In strains C3H and A yellow tissue is rare in periods 1 and 2. In periods 3 and 4 it increased in all the strains, although the pressure exerted by hyaline corpora lutea may destroy yellow tissue, as, for instance, in some of the mice of strain D. In strain AKA there occurred patches of yellow tissue in which the nuclei had been lost. In the New Buffalo strain it could be distinctly seen that the yellow cells may develop in the center of groups of light vacuolar cells which presumably gave origin to the pigmented cells. They may also be derived from smaller cuboidal interstitial gland cells. In strain A there were atretic follicles in which the theca interna cells were vacuolar and the membrana granulosa lutein-like.

Dilatation of Vessels, Hemorrhages and Hemorrhagic Pigment in the Interstitial Gland.—The yellowish brown pigment which develops in the course of time in the aging interstitial gland cells is presumably chemically similar to or identical with the endogenous pigment present in the corpora lutea. However, there may be admixed to this pigment in places yellowish hematogenous pigment, which some of the larger vacuolated interstitial gland cells seem to take up from the surrounding tissue. There are sufficient opportunities for the formation of such hematogenous pigment in various places in the ovary and especially also in the interstitial gland tissue. Not rarely one sees many enlarged blood vessels in which the slowing down of the blood stream provides an opportunity for the red blood corpuscles to escape from the vessels or for larger hemorrhages to occur in the interstitial gland. Thus in strain CBA yellow cells seem to contain some blood pigment in period 4. The sources of the extraneous blood pigment were ovarian, especially medullary hemorrhages and hemorrhagic cysts. There were dilated capillaries in different parts of the ovary. But conditions of this kind could be noted also in early periods. Thus in strain D enlarged

medullary vessels were found in period 1. In period 2 some blood pigment was present in vacuolar cells, which had presumably taken it up from the outside by phagocytosis. In the hilus an angioma-like network of vessels was found; in addition dilated capillaries and lymph vessels occurred in adenoma-like structures. Also in period 3 hyperemia could be noted in the interstitial gland. A heavy angioma-like body was found likewise in strain A in period 1. In period 4 a hemorrhagic cyst was observed in the interstitial gland. In addition a germinal cyst was hemorrhagic; the source of the hemorrhage in this instance was probably some dilated vessel surrounding the cyst. Similar dilatation of vessels and hemorrhages were found also in other strains, mainly in periods 3 and 4; but hematogenous pigment could be seen even as early as in period 1. In strain D there was observed in period 2 a body of polyhedral cells, the nature and origin of which could not be determined.

Relation of Corpora Lutea and Atretic Follicles to the Interstitial Gland.—There are strong indications that the theca interna of atretic follicles is one of the principal sources of the interstitial gland, but the membrana granulosa of atretic follicles, also, perhaps, takes part in the formation of the interstitial gland in some instances. Thus in strain C57 it seems that in the stage of atresia the granulosa, which may participate also in the rosette formation, may be included in interstitial gland tissue. In strain A the granulosa of atretic follicles seemed to undergo lutein-like changes and then to become incorporated in the interstitial gland. Moreover, in various strains pictures have been seen which suggest that regressing corpora lutea as well may become parts of the interstitial gland. A strong argument in favor of this view is the observation that occasionally areas of hyalinization are seen in the interstitial gland in strains in which the corpora lutea undergo hyaline or gelatinous degeneration. This was the case, for instance, in strain D in period 3. It was noticeable, too, in strain A during period 4. On the other hand, in strain C3H, in which hyalinization likewise occurs in corpora lutea, the interstitial gland was found free of such changes. In some respects one might therefore regard the interstitial gland as a reservoir into which various kinds of regressing or degenerative constituents of the ovary are pushed and deposited until in the end they disappear.

Change in Duct Formation with Increasing Age in Different Strains of Mice.—Two kinds of ducts have to be distinguished: (1) those that develop by growing out from the remnants of the wolffian body in the medulla; (2) those that dèvelop from the germinal epithelium covering the surface of the ovary by growing downward. The latter can be distinguished from the former in many cases by the fact that the subgerminal spindle cell tissue surrounds them in their downgrowth and

furnishes a lining for these germinal epithelial ducts. Another means of differentiation may be that at least in some instances the epithelial cells lining the medullary ducts may be higher, cylindric, in contrast to the flat cuboidal epithelium of the germinal epithelial ducts. In general the number of ducts which form increases with increasing age, and in addition various changes may take place in them. Thus the cells lining the ducts may become vacuolated, acquire yellow pigment and fill the lumens of the ducts. Or the ducts may become coiled, convoluted, and form adenoma-like structures, which on cross section may appear as a collection of alveoli. When these ducts come into contact with interstitial gland or with corpus luteum tissue, one gains the impression in many places that one has to deal not only with a contact between ducts and interstitial gland or lutein-like tissue but with an actual tendency of the ducts to transform into interstitial gland. In the ducts and in this real or apparent transitional tissue between ducts and interstitial gland, mitotic figures may occasionally appear as an indication of the growth processes which can take place in these tissues. All these changes tend to occur preferably in older mice during period 3 and especially during period 4. However, some uncertainty exists as to the interpretation of these pictures. Instead of an actual transformation of ducts one may have to deal merely with contact between interstitial gland and ducts in which the cells lining the ducts can become similar to interstitial gland cells. Moreover, in some instances one may have to deal not with real ducts but merely with imitation ducts. This appearance may be due to the growing down of the subcapsular spindle cells into the interstitial gland or lutein-like tissue. The spindle cells may surround strands of interstitial gland tissue, which thus may give the impression of ducts. Such an occurrence also might produce pictures simulating a transition between ducts and interstitial gland tissue.

In rather rare instances convoluted ducts may appear as early as in period 2. This occurred, for instance, in a mouse belonging to strain D. Also an adenoma-like structure developed in strain D in period 2. Furthermore, in a mouse of strain D, in period 2, medullary ducts were lined with cells which resembled those seen in ordinary sebaceous glands, and in general similar changes may take place in germinal epithelial ducts and in medullary ducts. Both germinal epithelial and medullary ducts may coil and subsequently present the appearance of adenoma. In all the strains the number of ducts increased and the usual further changes took place during periods 3 and 4.

Formation of Cysts.—There were two kinds of cysts, as there were two kinds of ducts, in the ovary: medullary cysts and epithelial germinal cysts. The former were derived from medullary epithelial structures, and they could be present as early as in periods 1 and 2, although this was not the usual occurrence. The germinal epithelial cysts were

derived as a rule from the germinal epithelial slits surrounding the ovary, which became dilated. The epithelium covering the ovary directly was usually higher than the epithelium lining the outer wall of the cyst, which was pressed flat. As stated, these cysts are not usually present in periods 1 and 2, although they did occur in some cases; for instance, in strain D a germinal epithelial cyst was noted in period 2. In strain A there was in period 4 a cyst filled with blood which was probably derived from dilated hyperemic vessels surrounding the cyst. Papillae consisting of central vessels surrounded by lutein-like interstitial gland tissue could protrude into medullary as well as into germinal cysts. Medullary cysts in places exerted pressure on the remainder of the ovary.

Transitions Between Ducts or Adenoma-like Structures and Interstitial Gland Tissue.—There is a sequence of changes which the ducts may undergo with the advancing age of the host. They first may coil and give rise to convolutions, which then can become adenoma-like. In the end either the ducts or the adenoma-like tissue may perhaps change to interstitial gland or to an intermediate lutein-like tissue. I have discussed already these apparent changes, as well as those which may take place in the ducts themselves, and have mentioned the differences in the interpretation of these changes. These alterations take place mainly in period 4 or to a more limited extent in period 3. There was one exception to this rule: In strain A a ductlike structure seemed to give origin to interstitial gland as early as in period 2.

Behavior of Subgerminal Spindle Cell Tissue.—Underneath the germinal epithelium a layer of spindle cell tissue is found. This tissue differs from resting spindle cell connective tissue by the great cellularity of the layer and by the absence or rareness of fibrils between the cells. The tissue resembles a similar layer found underneath the capsule in the adrenal gland of mice. In both adrenal glands and ovaries this tissue may send strands downward into the deeper structures of these glands, and during this process it may gradually become richer in connective tissue fibrils and thus may come to resemble ordinary connective tissue. In the adrenal cortex the subcapsular spindle cell tissue sends strands of spindle cells between the rows of cortical tissue as far down as the intermediate zone separating cortex and medulla. In the ovary the spindle cell tissue often envelops the ducts which develop from the germinal epithelium, and it envelops also parts of the germinal epithelial cysts; or the spindle cells may grow downward into the interstitial gland, thereby separating strands of interstitial gland cells from one another. In strain D the subgerminal spindle cell tissue was seen to penetrate also into gelatinous corpora lutea.

Mitotic Proliferation in Interstitial Gland and Ducts.—Mitotic figures appeared in various strains in structures in which they usually were not seen in mice. For instance, in strain CBA they were noted in period 4 in coiled ducts either of germinal epithelial or of medullary origin, also in transitional lutein-like interstitial gland tissue which may perhaps have been derived from these ducts. In strain D mitotic figures likewise were noted in ducts and in transitional tissue during period 3. In period 4 they appeared in adenoma-like structures derived from ducts and also in ducts of germinal epithelial and of medullary origin. In strain A they were noted in ducts and interstitial gland tissue during period 4. They were thus mostly seen in period 4, instead of in younger mice, but they did appear also in period 3; they seemed to be the expression of the increased activity of these structures in older mice and were perhaps connected with the apparent transition between ducts and interstitial gland tissue.

Penetration of Ducts and Interstitial Gland Tissue into the Fat Tissue Surrounding the Ovary.—In three strains it was observed that ducts and interstitial gland tissue, the latter sometimes assuming a lutein-like appearance, were able to penetrate through the surface of the ovary into the surrounding fat tissue. In every case this was observed only during period 4, therefore in old mice, and it was evidently related to the frequent abnormally strong development of these structures in mice of advanced age. In strain CBA germinal ducts which were coiled penetrated in some places into the surrounding fat tissue. Also in strain Old Buffalo ducts, lined with lightly staining vacuolar cells or with large yellow vacuolar cells, and in particular coiled ducts, including germinal ducts surrounded by lutein-like tissue, invaded the surrounding fat tissue. In strain C3H germinal ducts together with yellow interstitial gland tissue broke through the surface of the ovary into the surrounding fat tissue. In this connection mention might be made of another peculiarity which was noted in strain CBA during period 4, namely, the epithelial pearls which developed in ducts and especially in cystically dilated ducts, while other ducts were lined with flat epithelium. The pearls consisted of squamous epithelium surrounding concentric hyaline material. The changes mentioned in this paragraph and also in the following paragraph resembled very much conditions which may be seen during the formation and growth of tumors.

Hyalinization of Structures Other Than Corpora Lutea in the Ovaries of Mice During Various Periods of Life.—A continuous increase of hyalinization takes place in the successive periods of life. Hyalinization is as yet slight in period 1; it was observed in only a few mice in strain A in this period. It became much more widespread during periods 2 and 3. There was probably an increase in period 3 as compared with period 2, although the difference between these two

periods was not pronounced. It definitely increased in period 4 over periods 2 and 3. While the number of mice available for study at that time of life was relatively small in all the strains, the proportion of mice affected by hyalinization was greater during period 4 than during periods 2 and 3. Therefore with increasing age of mice the tendency toward hyalinization increases. In this respect conditions in the mouse correspond to those noted in the human species. There is a relation between the processes of hyalinization which take place in the corpora lutea and those in or around vessel walls. As we have seen, in corpora lutea hyalinization may begin around vessels, which then may be surrounded by a hyaline ring. But hyalinization of vessels does not have to be limited to the corpora lutea; it may occur also in the ovarian tissue outside the corpora lutea, and even the arteries in the tissue surrounding the ovaries may be affected by this process. However, that an actual connection exists between the hyalinization of vessels and that of corpora lutea, at least in some cases, is indicated also by the fact that in period 1 hyaline vessels were found only in strain A, in which the tendency to hyalinization of corpora lutea is strong. On the other hand, in many instances processes of hyalinization may appear in or around vessel walls even without the presence of hyalinized corpora lutea, and in strains in which hyalinization of corpora lutea is rare. Furthermore, hyalinization may affect structures other than corpora lutea. It is therefore probable that hyalinization of vessel walls may occur independently of hyalinization of corpora lutea but that in some instances this process occurring in corpora lutea may be associated with similar changes in or around the vessel walls. In sections stained with hematoxylin and eosin no difference can be detected between the hyaline deposits in vessel walls and those in corpora lutea. Differential staining of amyloid substance may not succeed in the ovary of the mouse, as indicated in earlier experiments, in which an attempt was made to differentiate amyloid from other hyaline substances in the adrenal gland of the mouse.

As stated already, hyalin may be deposited either around a vessel or in the media or the intima of the vessel. It was especially during period 4 in older mice that hyaline substance was discovered in the intima underneath the endothelium or in the media of the vessel in addition to perivascular hyaline substance. Hyaline material appeared not only around arteries but also around capillaries. In some cases the vessel walls could undergo more acute injury leading to necrosis, with or without the necrotic areas being subsequently incrusted with calcium salts. This occurred especially in vessels which were surrounded by hyaline-gelatinous corpus luteum tissue, which presumably exerted pressure on the walls of the vessels and thus interfered with their supply of oxygen or of foodstuffs. Such necrotic areas, on the

whole, were not frequent, but they were observed in period 2 as well as in period 3. While hyalinization was therefore quite common in arteries, the farther going changes, consisting in gelatinization and solution, which are so frequent in corpora lutea, seemed to be lacking in the vessel walls, which underwent in the mouse changes similar to those seen in the other species.

As stated processes of hyalinization were not limited to corpora lutea and blood vessels but affected also certain other structures. Thus in follicular atresia a hyaline membrane could be seen separating membrana granulosa and theca interna. Furthermore, instead of a layer of spindle cells lying underneath the germinal epithelium, this cellular layer could in places be replaced by a hyaline membrane. Likewise, instead of strands of spindle cells, extending from the subgerminal area downward, layers of hyaline material passed downward into the interstitial gland tissue in some instances and separated areas of interstitial gland from each other. Not only the vessel walls were surrounded by streaks of hyaline tissue but also ducts which develop in the ovaries with advancing age and in particular the medullary ducts. Lastly there may appear in ovaries in different places diffuse areas of dense hyaline material. They may be seen in various parts of the ovary, but perhaps they are more frequent in the medullary portion than elsewhere. These areas do not seem to be connected with the corpora lutea; they may perhaps develop around blood vessels, although this is not certain, at least not in all cases. Again, nutritional deficiencies may lead to necrosis in certain parts of the hyaline material, and such areas were surrounded by foreign body giant cells in some instances.

As to the mode of formation of these various hyaline deposits, the frequency with which they are observed around arterial walls suggests that they may be due to the deposition of material carried by the blood and passing from the blood vessels into the surrounding tissue, where it perhaps undergoes further chemical or physical changes. However, this can hardly be taken to be the only mode of development of these hyaline structures if one considers the fact that hyalin is also deposited around ovarian ducts and in other sites where it cannot well represent a modified transudate passing through the vessel wall to the outside. Instead in some instances it may perhaps be due to a hyaline transformation of connnective tissue fibers.

In regard to the question whether a strain could be genetically predisposed to hyalinization outside of that seen in corpora lutea, it is significant that no hyalinization was noted in strain C. Hyalinization was rare also in strain CBA, but it appeared in this strain in period 4. In strain C57 it occurred in periods 2, 3 and 4, although the corpora lutea showed only a slight tendency to hyalinize. While in the case of this strain there was apparently no parallelism between hyalinization

of corpora lutea and that of other structures, in general in strains which exhibited a greater tendency toward hyalinization of the corpora lutea, there seemed also to be a greater tendency toward hyalinization of other structures. Thus in strains AKA and D and also in strain C3H there was generally much hyalinization of both kinds, whereas in strain C and in strain CBA there was only a slight tendency toward both these types of hyalinization. However, the data available so far make possible only tentative conclusions in this respect.

SUMMARY

In all the strains of mice the interstitial gland of the ovary increased in size with the increasing age of the mice; in very young mice it was as yet rudimentary. The interstitial gland cells underwent marked changes especially in periods 3 and 4, when the cells tended to become more or less vacuolated and to accumulate yellow or brown pigment. The interstitial gland tissue seemed to be derived mainly from the theca interna of atretic follicles, but there were indications that regressing corpora lutea, also, or even the membrana granulosa of atretic follicles could be incorporated in the interstitial gland. The fact that hyaline changes occurred in the interstitial gland in strains in which similar changes took place in corpora lutea also supports the view that regressing corpora lutea may become a part of the interstitial gland. In strain C57 black the interstitial gland had a tendency to form syncytia and rosettes, and the pigment in this tissue developed at an early stage; similar changes occurred in the adrenal gland of strain C57 black. In some strains the corpora lutea, especially the hyalinized ones, exerted pressure on the interstitial gland and helped to destroy it.

There occurred mainly in periods 3 and 4, but sometimes as early as in periods 1 and 2, hemorrhages in the ovaries, especially in the interstitial gland; these occasionally were enclosed in cystlike spaces and were associated with much dilated capillaries. The vacuolated interstitial gland cells could take up red blood cells or hematogenous pigment. In 1 case the presence of dilated vessels adjacent to a germinal epithelial cyst seemed to have resulted in the filling of the cyst with coagulated blood. Also hemangioma-like structures were found in periods 1 and 2 in strains A and D.

With advancing age the germinal epithelial and the medullary ducts increased in number. As a rule, they became very numerous in periods 3 and 4. In period 3, and still more so in period 4, the ducts underwent further changes, particularly coiling, formation of adenoma-like structures, vacuolation of duct cells and apparent or real transitions leading to lutein-like interstitial gland tissue. Occasionally an adenoma-like structure was found as early as in period 2. Cysts also increased in number with increasing age, although some cysts, especially those of

medullary origin, were found in periods 1 and 2. The medullary cysts were derived from medullary ducts, and the germinal epithelial cysts developed as a result of dilatation of the germinal epithelial slits covering the ovary.

The layer of subgerminal spindle cell tissue corresponding to similar tissue in the subcapsular layer of the adrenal gland formed a sheet around germinal epithelial ducts and cysts and thus helped to distinguish these structures from ducts and cysts of medullary origin. These spindle cells also were most prominent in period 4, but they are noticeable at earlier times.

With increasing age, mainly in period 4 but to some extent also in period 3, certain additional growth phenomena were seen which were not unlike changes taking place in early tumor formation. Mitotic figures appeared in medullary as well as in germinal epithelial ducts, in adenoma-like structures, in lutein-like tissue and in fully developed interstitial gland tissue. These tumor-like processes have been seen so far only in some of the strains. Ducts accompanied by lutein-like interstitial gland or perhaps interstitial gland alone could penetrate through the surface of the ovary into the surrounding fat tissue.

Processes of hyalinization occurred not only in or around arteries and capillaries but also in various other structures of the ovaries. They increased in frequency with increasing age and were partly associated with processes of hyalinization which took place in corpora lutea. It is probable that the hyaline changes which were noted around blood vessels and in the walls of blood vessels were of the same kind as those which occur in blood vessels generally with increasing age.

EFFECTS OF FOLIC ACID ANTAGONISTS INOCULATED IN EMBRYONATED EGGS

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THE ANTAGONISTS of folic acid that have been most carefully studied are analogues of four main types. In the first type the substituents of the pteridine ring are altered. In the second a substituent is introduced on the amino group of the para-aminobenzoylglutamic acid portion of the molecule. In the third a different amino acid is substituted for glutamic acid. Combinations of these variations compose the fourth type.

The following observations are concerned with the effects on chick embryos of three folic acid antagonists belonging to types 1, 2, and 4. These studies were made by a new technic for evaluating the hemopoietic activity of various substances in the chick embryo. Some of the findings have appeared in a preliminary note.[1]

MATERIALS AND METHODS

Chick embryos 6 to 8 days old were inoculated via the yolk sac with three folic acid antagonists (4-aminopteroylglutamic acid, N^{10}-methylpteroic acid, (methyl-4-aminopteroylglutamic acid) alone or with folic acid,[2] liver injection or vitamin B_{12}[3] in varying amounts. All the substances except injection U.S.P. (solution liver extract purified,* 15 U.S.P. units per cubic centimeter) were dissolved in distilled water. The embryos were incubated at 35C. All deaths occurring in the first forty-eight hours were considered post-traumatic and discarded from the study.

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1. Wagley, P. F., and Morgan, H. R.: Bull. Johns Hopkins Hosp. **83**:275-278, 1948.

2. The 4-aminopteroylglutamic acid (lot 7-7843C), N^{10}-methylpteroic acid (lot 7-7623A), methyl-4-aminopteroylglutamic acid (lot 7-8185) and folic acid (as synthetic pteroylglutamic acid) were obtained through the Lederle Laboratories, Pearl River, N. Y.

3. Vitamin B_{12} was provided by Dr. A. Gibson, of Merck & Co., Inc., Rahway, N. J.

Blood was obtained for cell counts and hemoglobin determinations by removing the shell over a large allantoic vessel previously visualized by transillumination. The shell membrane was kept intact. A drop of liquid petrolatum was placed on the intact membrane to improve visualization. Venipuncture was done with a 27 gage needle and a 0.25 cc. or 0.5 cc. syringe. Potassium-ammonium oxalate mixtures added to such blood did not prevent a coagulum from forming. Cell counts were therefore made immediately, using isotonic sodium chloride solution or Gower's solution as a diluent. Hemoglobin determinations were made with an Evelyn colorimeter.

Yolk sacs were fixed in 10 per cent acetic acid–Zenker's solution, and microscopic sections were stained with eosin–methylene blue.[4]

TABLE 1.—*Effect of 4-Aminopteroylglutamic Acid on Chick Embryos*

Series	4-Aminopteroylglutamic Acid, Mg.	Survival of Embryos, Age in Days								Microscopic Appearance of Yolk Sac Blood Islets
		7	8	9	10	11	12	13	14	
1	1.0	3/3*	3/3	3/3	0/3	...	...	...	...	
2	0.1	4/4	4/4	4/4	2/4	0/4	...	...	...	
3	0.01	5/5	5/5	5/5	5/5	2/5	0/5	...	...	Diminution in size and number of blood islets; pyknosis, karyolysis and karyorrhexis of nuclei
4	0.005	10/10	10/10	10/10	10/10	7/10	6/10	4†/10	0/10	Diminution in size and number of blood islets; pyknosis, karyolysis and karyorrhexis of nuclei
5	0.001	10/10	10/10	10/10	10/10	10/10	10/10	8/10	7/10	Diminution in size and number of blood islets; pyknosis, karyolysis and karyorrhexis of nuclei
6	Distilled water 1.0 cc.	5/5	5/5	5/5	5/5	5/5	3*/5	3/5	3/5	Normal

* The numerator is the number of embryos that survived; the denominator, the number given injections.

† Two embryos were killed for study. The average cell count and hemoglobin determination are given in the text.

EXPERIMENTS

EXPERIMENT 1.—Five series of 7 day chick embryos were inoculated with various amounts of 4-aminopteroylglutamic acid. A sixth series received only an inoculum of distilled water as a control. The observations on survival and the microscopic observations on the blood-forming tissues are summarized in table 1.

There appeared to be a shortening of the survival time with an increase of the amount of the antagonist. In addition, histologic study of the blood islets of the yolk sac revealed a decrease in their number, size and cellularity. The hemopoietic cells that were still present showed marked pyknosis and fragmentation of the nuclei. Other nuclei showed decreased basophilia. These changes are illustrated in figure 1, which shows a blood islet of the yolk sac of a 12 day old chick embryo which had been inoculated on the seventh day of life with 0.005 mg. of 4-aminopteroylglutamic acid. For comparison, a normal

4. Miss Lillian M. Leavitt, of the Mallory Institute of Pathology, prepared the microscopic sections.

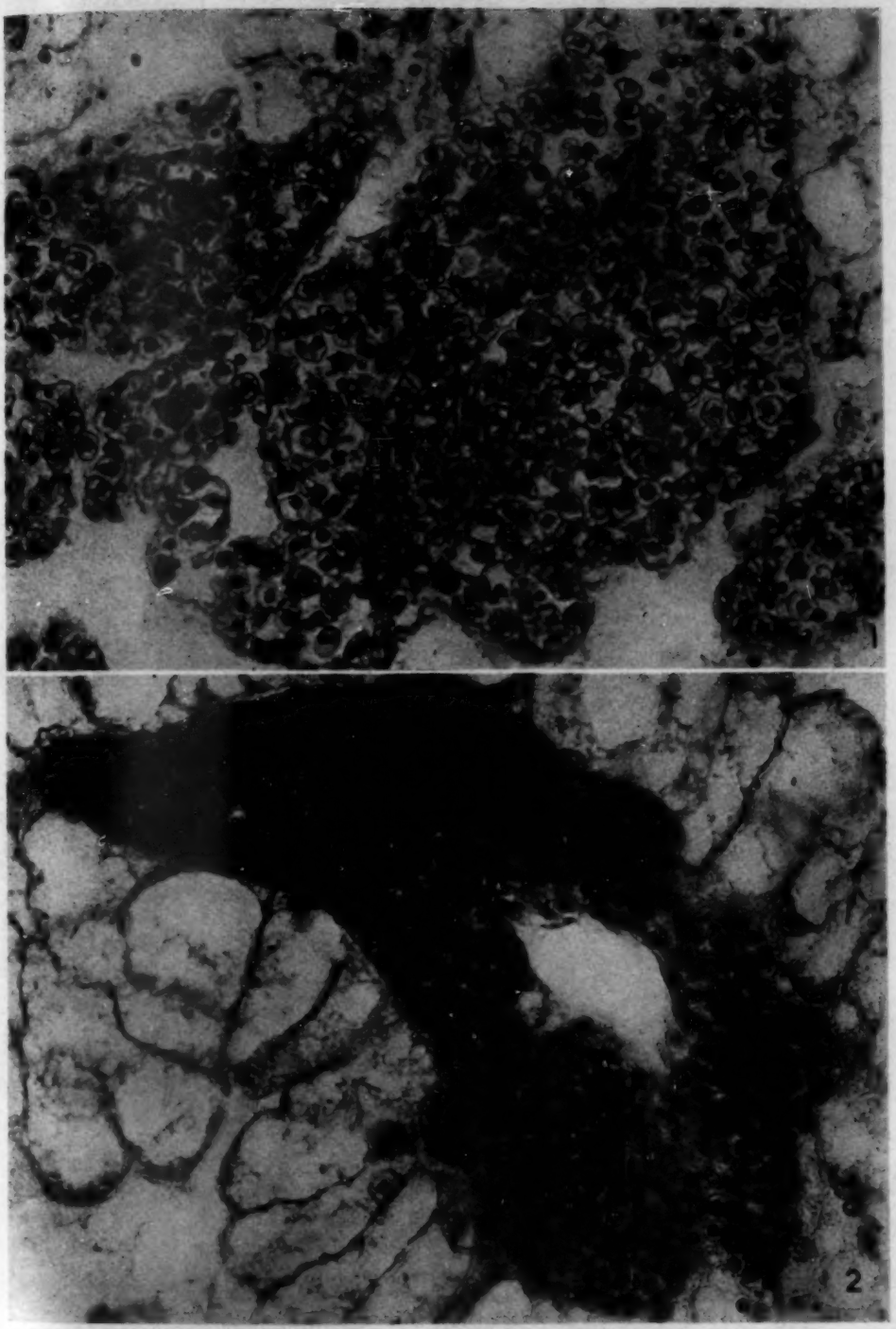

Fig. 1.—Yolk sac blood islet of a 12 day old chick embryo inoculated five days previously with 0.01 mg. of 4-aminopteroylglutamic acid. There is marked loss of cellularity (many of the cells visible are intravascular) with pyknosis and karyolysis of the nuclei. × 333.

Fig. 2.—Yolk sac blood islet of a normal 12 day old chick embryo. × 333.

yolk sac blood islet of a 12 day chick embryo of the control group of this experiment (series 6) is shown in figure 2.

Cell counts and hemoglobin determinations were made on the blood of 2 embryos of series 4 and 2 embryos of series 6 at the age of 13 days. The total blood cell counts of the 2 receiving 0.005 mg. of 4-aminopteroylglutamic acid six days previously (series 4) averaged 1,300,000 per cubic millimeter, and the hemoglobin levels averaged 5.1 Gm per hundred cubic centimeters of blood. The corresponding values obtained on the same day in the controls (series 6) aver-

TABLE 2.—*Influence of Certain Hemopoietic Substances on the Effects of 4-Aminopteroylglutamic Acid in Chick Embryos*

Series	Drug	Dose	Age at Inoculation in Days	Survival of Embryos, Age in Days								Microscopic Appearance of Yolk Sac Blood Islets
				7	8	9	10	11	12	13	14	
1	4-aminopteroylglutamic acid	0.01 mg.	7	10/10†	10/10	10/10	9/10	6*/10	6/10	6/10	5/10	Slight diminution in cellularity of blood islets
	Folic acid	12.5 mg.	6									
	Liver injection U. S. P.	3.75 units	6									
2	4-aminopteroylglutamic acid	0.01 mg.	7	9/9	9/9	9/9	9/9	6*/9	6/9	6/9	6/9	Slight diminution in cellularity of blood islets
	Folic acid	12.5 mg.	6									
3	4-aminopteroylglutamic acid	0.01 mg.	7	12/12	12/12	12/12	9/12	5*/12	2/12	2/12	1/12	Diminution in size and number of blood islets; pyknosis, karyolysis and karyorrhexis of nuclei
	Liver injection U. S. P.	3.75 units	6									
4	4-aminopteroylglutamic acid	0.005 mg.	8	.....	3/3	3/3	3/3	3/3	2/3	2/3	0/3	Diminution in size and number of blood islets; pyknosis, karyolysis and karyorrhexis of nuclei
	Vitamin B_{12}	0.005 mg.	8									
5	4-aminopteroylglutamic acid	0.005 mg.	8	.....	1/1	1/1	1/1	1/1	1/1	1/1	1/1	Diminution in size and number of blood islets; pyknosis, karyolysis and karyorrhexis of nuclei
	Vitamin B_{12}	0.011 mg.	8									
6	4-aminopteroylglutamic acid	0.01 mg.	7	11/11	11/11	11/11	10/11	5*/11	5/11	2/11	0/11	Diminution in size and number of blood islets; pyknosis, karyolysis and karyorrhexis of nuclei

* Three embryos were killed for study.
† The numerator is the number of embryos that survived; the denominator, the number injected.

aged 1,800,000 per cubic millimeter and 6.5 Gm. per hundred cubic centimeters of blood.

About 0.01 to 0.005 mg. of 4-aminopteroylglutamic acid seemed to be the smallest amount consistently giving detectable alteration in the survival time and this histologic appearance of the blood islets under the conditions of these experiments.

EXPERIMENT 2.—The purpose of experiment 2 was to determine whether or not folic acid, liver injection U.S.P. (solution liver extract, purified,* 15 U.S.P. units per cubic centimeter) or vitamin B_{12} would protect chick embryos from the effects of 4-aminopteroylglutamic acid. As shown in table 2, 6 series of embryonated eggs 7 to 8 days of age were inoculated with 0.01 or 0.005 mg. of the folic acid antagonist. In series 1, 2 and 3 the embryos were inoculated with liver injection U. S. P. and folic acid, in the amounts indicated, twenty-

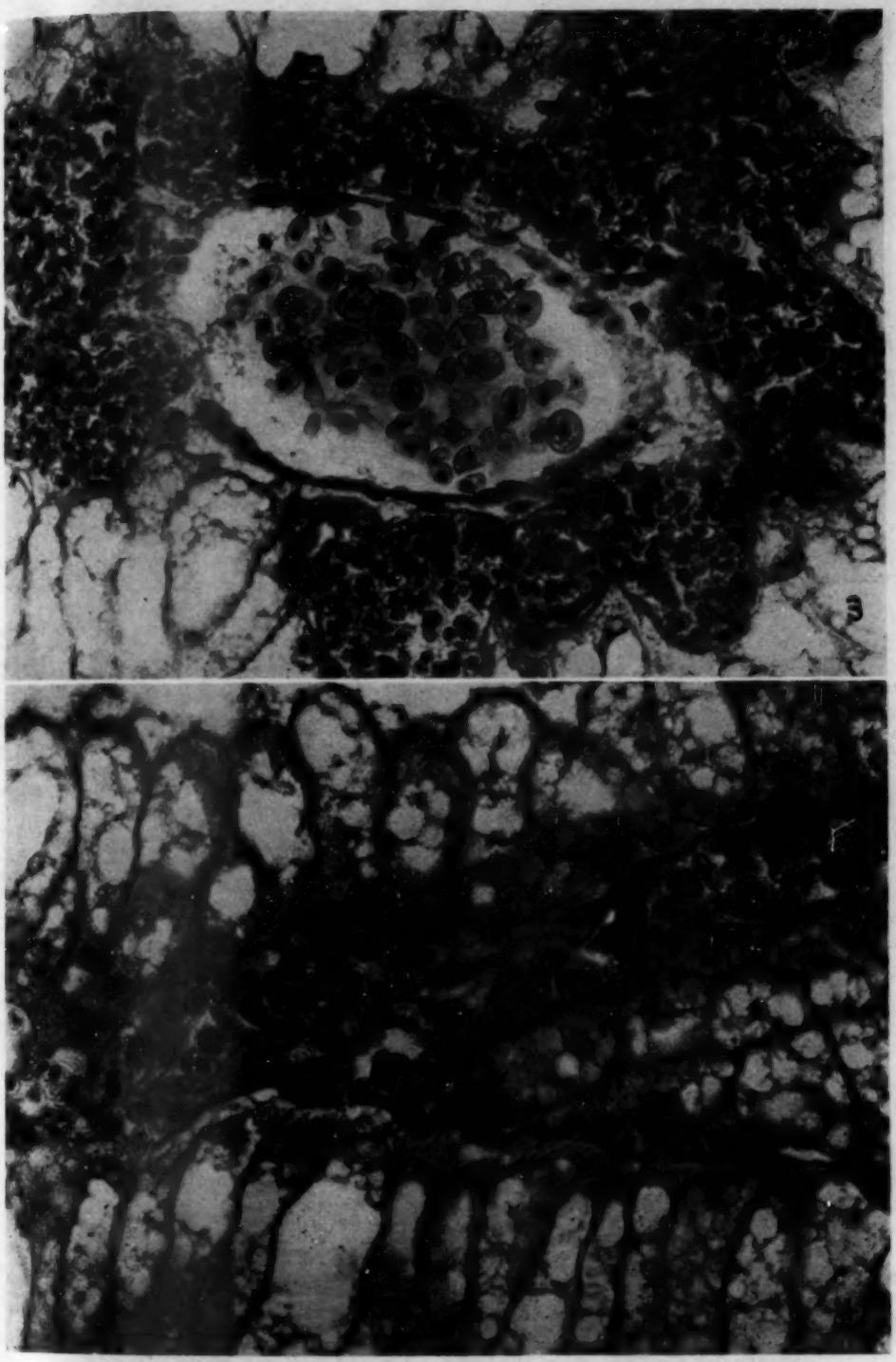

Fig. 3.—Yolk sac blood islet of an 11 day old chick embryo inoculated five days previously with 12.5 mg. of folic acid and four days previously with 0.01 mg. of 4-aminopteroylglutamic acid. There is diminution in the cellularity of the blood islet, but not as marked changes occurred as with the antagonist alone. × 333.

Fig. 4.—Yolk sac blood islet of a 12 day old chick embryo previously inoculated on the sixth day of life with 3.75 U.S.P. units (injectable) of liver extract and on the seventh day with 0.01 mg. of 4-aminopteroylglutamic acid. There is marked diminution of the cellularity with loss of basophilia of the nuclei. × 333.

four hours prior to the inoculation of the antagonist to allow the substances to diffuse through the tissues of the embryo before the 4-aminopteroylglutamic acid was injected. Series 4 and 5 consisted of 4 embryonated eggs each. Each embryo received 0.005 mg. of the antagonist and 0.005 or 0.011 mg. of vitamin B_{12} simultaneously.

Although only folic acid, in the amounts employed, seemed to prevent a shortening of the survival time, the number of eggs used does not permit the definite conclusion that vitamin B_{12} and liver injection U. S. P. did not protect the embryo. However, histologic studies revealed only slight diminution of the cellularity of the blood islets of embryonated eggs inoculated with folic acid in 12.5 mg. amounts prior to the injection of 0.01 mg. of 4-aminopteroylglutamic acid (series 2). Changes in the remaining nuclei were rare and not impressive. Figure 3 illustrates a blood islet from an 11 day old embryonated egg of this series.

By contrast, the blood islets of the embryos receiving liver injection U. S. P. or vitamin B_{12} as well as the antagonist (series 3, 4 and 5) showed histologic

TABLE 3.—*Effects of N^{10}-Methylpteroic Acid and Methyl-4-Aminopteroylglutamic Acid on Chick Embryo*

Series	Drug	Dose	Age at Inoculation in Days	Survival of Embryos, Age in Days									Microscopic Appearance of Yolk Sac Blood Islets
				6	7	8	9	10	11	12	13	14	
1	N^{10}-methylpteroic acid	20 mg.	6	8/1*	8/8	8/8	8/8	7/8	6/8	4†/8	4‡/8	....	Normal
2	Distilled water	0.5 cc.	6	5/5	5/5	5/5	5/5	5/5	5/5	3†/5	3/5	3/5	Normal
3	Methyl-4-aminopteroyl-glutamic acid	0.01 mg.	8	...	...	11/11	11/11	11/11	11/11	11/11	7/11	6/11	Diminution in size and number of blood islets; some fragmentation of nuclei
4	Methyl-4-aminopteroyl-glutamic acid Folic acid	0.01 mg. 0.1 mg.	8 8	...	...	12/12	12/12	12/12	12/12	11/12	10/12	10/12	Slight diminution in cellularity of blood islets; occasional fragmentation of nuclei

* The numerator is the number of embryos that survived; the denominator, the number receiving injections.
† Two embryos were killed for study.
‡ The experiment was terminated.

changes indistinguishable from those occurring in embryos inoculated only with the antagonist (series 6). The amount of purified antipernicious anemia liver extract injected into each egg contained 3.75 U. S. P. units (injectable), i.e., activity the equivalent of 2.7 micrograms of vitamin B_{12}. Figure 4 shows a blood islet of a 12 day old embryonated egg of series 3 inoculated on the sixth day with 3.75 U. S. P. units (injectable) and on the seventh day with 0.01 mg. of the antagonist. There is diminution in the cellularity of the blood islet, with nuclear changes, as shown in the higher magnification of figure 5. Pale-staining nuclei were prominent, and several were visible only as fragments or basophilic granules. The blood islet of the chick embryo that was inoculated on the eighth day with both 0.011 mg. of vitamin B_{12} and 0.005 mg. of 4-aminopteroylglutamic acid died at the end of the thirteenth day and showed diminution in the cellularity of the blood islets, with pyknosis, karyolysis and karyorrhexis of the nuclei (fig. 6).

EXPERIMENT 3.—Two other inhibitors of folic acid were studied, as indicated in table 3. Four series of embryonated eggs were inoculated. In series 1 each embryo was given 20 mg. of N^{10}-methylpteroic acid on the sixth day. Hemoglobin determinations and blood cell counts made seven days later showed an average level

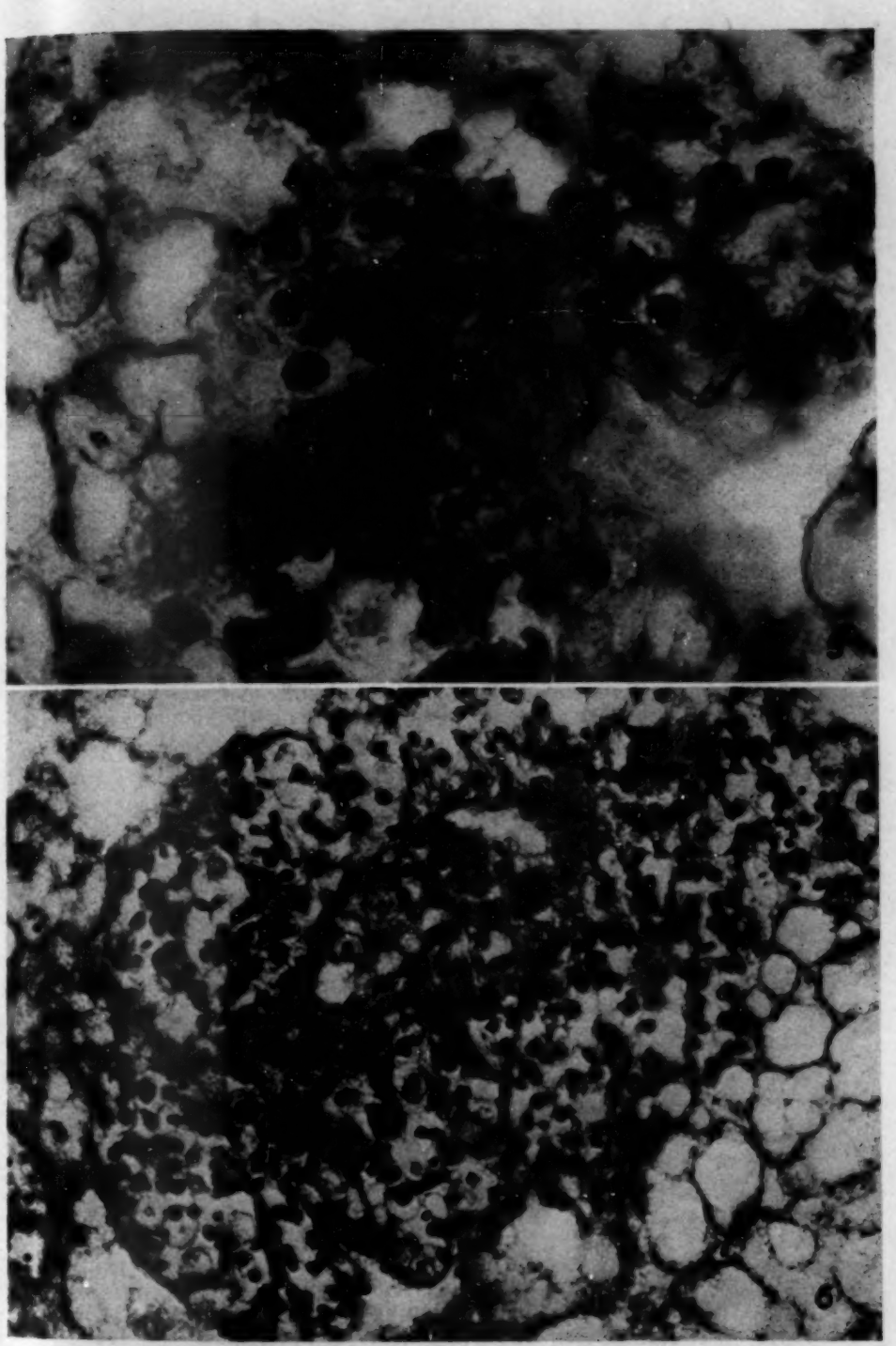

Fig. 5.—Higher magnification of a section of a yolk sac blood islet illustrating the fragmentation and karyolysis of the nuclei following injection of 0.01 mg. of 4-aminopteroylglutamic acid. This occurred irrespective of the presence of 3.75 U.S.P. units (injectable) of liver extract or 0.011 mg. of vitamin B_{12}. × 1500.

Fig. 6.—Yolk sac blood islet of a 13 day old chick embryo inoculated five days previously with 0.005 mg. of 4-aminopteroylglutamic acid and 0.011 mg. of vitamin B_{12}. There is marked diminution in cellularity with pyknosis, karyolysis and karyorrhexis of nuclei. × 333.

of 5.5 Gm. of hemoglobin per hundred cubic centimeters of blood and 1,330,000 cells per cubic millimeter. The corresponding figures for the control series (series 2), inoculated only with distilled water, were 5.3 Gm. of hemoglobin and 1,340,000 cells. The blood islets of the chick embryos of series 1 and 2 showed no definite variation in comparison with the normal seven days after inoculation with N^{10}-methylpteroic acid.

Series 3 and 4 consisted of embryonated eggs inoculated on the eighth day with 0.01 mg. of methyl-4-aminopteroylglutamic acid; those of series 4 also received 0.1 mg. of folic acid simultaneously. The yolk sac blood islets of embryos of both series showed some diminution of size and number but such alterations were much less than those occurring with comparable doses of 4-aminopteroylglutamic acid. The inoculation of 0.1 mg. amounts of folic acid seemed to diminish these changes. Only occasional fragmentation of the nuclear structure was observed.

COMMENT

Several folic acid inhibitors have been described.[5] Martin and associates [5a] were the first to describe a displacing agent (α(-)methyl folic acid) for pteroylglutamic acid, which they demonstrated by using Streptococcus faecalis (lactis) R assay technic. Seeger and associates [5b] reported the synthesis of another folic acid analogue and antagonist, later referred to as 4-aminopteroylglutamic acid. By feeding a chemically unidentified folic acid antagonist to weanling female albino rats of the Wistar strain, Franklin and co-workers [6] produced granulocytopenia, leukopenia and anemia within a period of three weeks. The bone marrow of a rat with a severe antagonist-induced syndrome showed an increase in the proportion of nucleated red blood cells. Deeply staining blast cells of the erythroid series were seldom seen in the controls but were numerous in the marrow of the animals receiving the antagonist. Few mature granulocytes were found in the marrow of the latter group of rats. Other characteristic signs of the deficiency syndrome were loss of weight, unkempt, ruffled fur, encrustation of the vibrissae with red pigment, severe diarrhea, ulcerations of the oral cavity and gingivitis. Administration of more than 0.3 mg. of pteroylglutamic acid per gram of antagonist prevented these changes. Administration of 100 mg. of pteroylglutamic acid per 10 Gm. of antagonist caused rapid recovery, with normal appearance, growth rates and blood picture within a few weeks. Following recovery, the animals showed unexplained splenomegaly. Asenjo [7] has reported that splenic infarcts occur in rats on a diet deficient in folic acid.

5. (*a*) Martin, J. G.; Tolman, L., and Moss, J.: Arch. Biochem. **12**:318, 1947. (*b*) Seeger, D. R.; Smith, J. M., Jr., and Hultquist, M. E.: J. Am. Chem. Soc. **69**:2567, 1947. (*c*) Daniel, L. J.; Norris, L. C.; Scott, M. L., and Heuser, G. F.: J. Biol. Chem. **169**:689, 1947. (*d*) Wooley, D. W., and Pringle, A.: ibid. **174**:327, 1948.

6. Franklin, A. L.; Stokstad, E. L. R.; Belt, M., and Jukes, T. H.: J. Biol. Chem. **169**:427, 1947.

7. Asenjo, C. F.: Federation Proc. **7**:144, 1948.

Franklin and associates [8] have also produced slow growth, anemia and leukopenia in both mice and chicks with a chemical antagonist of pteroylglutamic acid. Such signs were reversible. However, large amounts of 4-aminopteroylglutamic acid (1 milligram per kilogram of diet) produced death within a few days in mice, and this effect was not prevented by as much as 100 mg. of pteroylglutamic acid per kilogram of diet. Welch and co-workers [9] have produced diminished growth rate, severe anemia, patchy alopecia and profuse diarrhea in a pig by feeding a purified diet, succinylsulfathiazole and a folic acid inhibitor. Reticulocytosis and rises of hemoglobin level and red cell count followed the changing of the diet to one containing the extrinsic factor.

Daniel and associates [5c] have demonstrated that several synthetic pterins possess marked antibacterial activity which is antagonized competitively by folic acid. Such pterins have the unique capacity of inhibiting bacteria which synthesize folic acid as well as those requiring the preformed vitamin. Edwards and co-workers [10] have described an experiment in which Str. faecalis utilization of folic acid was inhibited by a derivative of benzimidazole. Hutchings and co-workers [11] have reported pteroylaspartic acid to be an effective inhibitor of the utilizing of pteroylglutamic acid for growth by Lactobacillus casei, Str. faecalis R and the chick but not to be an effective inhibitor of such utilization by the rat. In the chick the inhibitor antagonized the growth effects of pteroylglutamic acid in a ratio of 500 to 1 by weight. Hall [12] has reported that quinoxaline can act as an inhibitor of the growth of Streptococcus lactis R and that pteroylglutamic acid can reverse the inhibition. Emerson and Mushett [13] have observed that in rats maintained on a purified diet containing 1 per cent sulfathiazole and all identified vitamins except pteroylglutamic acid, granulocytopenia and leukopenia developed. Treatment with either purified liver extracts or pteroylglutamic acid resulted in prompt restoration of normal numbers of white cells. An increase of the number of reticulocytes that was considered significant was also reported. Swenseid and co-workers,[14] during a study of a group of pteroylglutamic analogues and pterins, observed the development of leukopenia and/or anemia within a fourteen day period in weanling rats receiving purified diets containing succinylsulfathiazole. The pteroylglutamic acid analogues produced leukopenia and anemia, but the pterins had a preferential effect on the leukocytes in those particular experimental

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9. Welch, A. D., and others: Proc. Soc. Exper. Biol. & Med. **65**:364, 1947.

10. Edwards, P. C.; Starling, D.; Mattocks, A. M., and Skipper, H. E.: Science **107**:119, 1948.

11. Hutchings, B. L., and others: J. Biol. Chem. **170**:323, 1947.

12. Hall, D. A.: Biochem. J. **41**:294, 1947.

13. Emerson, G. A., and Mushett, C. W.: Federation Proc. **7**:285, 1948.

14. Swenseid, M. E., and others: Federation Proc. **7**:299, 1948.

conditions. Minnich and Moore [15] have produced normocytic anemia, leukopenia and thrombopenia, with hypoplastic marrows, in guinea pigs by daily intramuscular injection of 0.5 to 5.0 mg. of 4-aminopteroylglutamic acid. Neither liver extract nor folic acid, in the doses used, prevented the development of anemia, though folic acid did seem to prevent leukopenia and thrombopenia. Farber and associates [16] have reported that remissions were induced in acute leukemia with folic acid antagonists, pteroylaspartic acid and 4-aminopteroylglutamic acid.

A new method of studying the effects of folic acid antagonists, folic acid, liver extract and vitamin B_{12} is described. With this technic, 4-aminopteroylglutamic acid was found to be the most effective of the three antagonists used. The survival time of the inoculated embryos decreased as the dosage of the antagonist inoculated increased. With effective concentrations, detectable changes occurred in the level of hemoglobin and the total cell count. The cytologic changes in the blood islets of the yolk sac were marked. They were characterized by a decrease in both the number of the blood islets and their individual cellularity. The remaining nuclei showed pyknosis, karyolysis and karyorrhexis.

This effect, as well as that of the less active methyl-4-aminopteroylglutamic acid, appeared altered by injections of folic acid. However, liver injection U. S. P. and vitamin B_{12}, in the doses employed, did not alter the apparent effect of the 4-aminopteroylglutamic acid. N^{10}-methylpteroic acid in relatively larger doses than the other antagonists caused no detectable changes in the hemoglobin level or the cell count of the blood. The blood islets of the yolk sacs of the embryonated eggs inoculated with it appeared essentially normal seven days after the injection.

SUMMARY

A new technic has been employed for the study of folic acid inhibitors. By this technic it was shown that 4-aminopteroylglutamic acid caused marked changes in the hemopoietic tissue of embryonated eggs comparable to those described in other species. The blood islets of the yolk sac showed diminution in number and size with decreased cellularity. The remaining nuclei showed pyknosis, karyolysis and karyorrhexis. This effect was less marked in the presence of relatively large amounts of folic acid. In the dose employed, liver injection U. S. P. (solution liver extract purified,* 15 U. S. P. units per cubic centimeter) and vitamin B_{12} did not alter the effects of 4-aminopteroylglutamic acid in the yolk sac blood islets. N^{10}-methylpteroic acid in relatively large doses had no appreciable effect. Methyl-4-aminopteroylglutamic acid caused much less change in the blood islets than did comparable doses of 4-aminopteroylglutamic acid.

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16. Farber, S., and others: New England J. Med. **238**:787, 1948.

CHOLESTEROL OF THE HUMAN ADRENAL GLAND

Its Significance in Relation to Adrenal Function and Structure

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PREVIOUS investigations of the cholesterol of the adrenal gland [1] have led authors to conclude that, in general, the adrenal cholesterol levels were low in persons who died of severe infections, moderately low in chronic debilitating and maligant diseases and high in people who died of cardiovascular and/or renal disorders.

Recent progress in knowledge of the function of the adrenal gland has revealed certain facts, listed below, that facilitate the interpretation of the previous findings and prompt a reevaluation of the adrenal cholesterol in regard to its functional significance and its level in normal and in diseased states.

1. Animals that are subjected to nonspecific types of acute stress have a rapid fall of the adrenal cholesterol, provided the hypophysis of the experimental animal is intact.[2]

2. An extract of the anterior lobe of the pituitary gland containing the adrenocorticotropic principle when administered to animals causes a decrease in the cholesterol of the adrenal gland accompanied by changes similar to those that occur when adrenal cortex extracts are given.[3]

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston.

1. (*a*) Chauffard, A.; Laroche, G., and Grigaut, A.: Compt. rend. Soc. de biol. **73**:23, 1912; (*b*) **76**:529, 1914. (*c*) Fex, J.: Biochem. Ztschr. **104**:82, 1920. (*d*) Kohno, T.: Folia endocrinol. japan. **4**:60, 1928; (*e*) **4**:85, 1929.

2. Sayers, G.; Sayers, M. A.; Fry, E. G.; White, A., and Long, C. N. H.: Yale J. Biol. & Med. **16**:361, 1944. Long, C. N. H.: Recent Progress in Hormone Research, New York Academic Press, Inc., 1947, vol. 1, pp. 99-117.

3. Sayers, G.; Sayers, M. A.; White, A., and Long, C. N. H.: Proc. Soc. Exper. Biol. & Med. **52**:200, 1943. Sayers, G.; Sayers, M. A.; Liang, T., and Long, C. N. H.: Endocrinology **38**:1, 1946. Jensen, H., and Gratton, J. F.: Am. J. Physiol. **128**:270, 1940. Tyslowitz, R., and Astwood, E. B.: ibid. **136**:22, 1942. White, A., and Dougherty, T. F.: Ann. New York Acad. Sc. **46**:859, 1946.

3. In man the administration of the anterior pituitary extract containing the adrenocorticotropic factor causes an increased excretion of "cortins" and 17-ketosteroids.[4]

Two important features of adrenal cholesterol are apparent; it fluctuates widely and rapidly, depending on the exogenous and endogenous stimuli to which the organism has been subjected, and it appears to play an important role in the biochemical reactions of the adrenal cortex.

Therefore, we have attempted to define the range of the normal human adrenal cholesterol with emphasis on the fact that adrenal glands may be regarded as normal only when they are obtained from healthy persons who have died suddenly and who at autopsy show no pathologic changes other than those caused by the fatal agent. We have also determined the cholesterol content of adrenal glands of persons suffering from cardiac, renal, infectious, hepatic, cancerous and endocrine diseases and have correlated the findings with published data on the urinary excretion of "cortin-like" compounds [5] and 17-ketosteroids.[6] In correlating adrenal cholesterol levels with disease states, greater significance is attached to the findings after sudden death, since nonspecific changes in the adrenal gland from chronic illness, infections and therapeutic measures are at a minimum.

Tissue sections of the same adrenal glands were examined, and the histologic changes accompanying decreased and increased amounts of cholesterol are described. The latter group includes such lesions as "focal and nodular hyperplasia" and small adrenal adenomas. The functional significance and the genesis of the lesions associated with increased amounts of cholesterol are postulated on the basis of (1) the adrenal response to stress, (2) a comparison with the hyperplasia that occurred in 4 proved cases of hyperadrenocorticism and (3) the experimental data concerning the depletion and the accumulation of cholesterol in adrenal cortices.

MATERIALS AND METHODS

The materials and the methods used in this study were similar to those of a previous study.[7] Special histochemical methods, such as staining with phenyl-

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5. (*a*) Talbot, N. B.; Albright, F.; Saltzman, A.; Zygmuntowicz, A., and Wixom, R.: J. Clin. Endocrinol. **7**:331, 1947. (*b*) Venning, E. H., and Browne, J. S. L.: ibid. **7**:79, 1947. (*c*) Daughaday, W. H.; Jaffe, H., and Williams, R. H.: ibid. **8**:66, 1948. (*d*) Shipley, R. A.; Dorfman, R. I.; Buchwald, E., and Ross, E.: J. Clin. Investigation **25**:673, 1946.

6. Salter, W. T.; Cahen, R. L., and Sappington, T. S.: J. Clin. Endocrinol. **6**:52, 1946. Bruger, M.; Rosenkrantz, J. A., and Lowenstein, B. E.: Am. J. M. Sc. **208**:212, 1944. Venning and Brown.[5b]

7. Rogers, W. F., Jr., and Williams, R. H.: Arch. Path. **44**:126, 1947.

hydrazine and examining by means of ultraviolet rays, were not used because of the previously reported nonspecificity of these methods.[7]

ADRENAL CHOLESTEROL

The adrenal cholesterol values of the 11 cases that fulfilled the criteria of normality are given in table 1. They relate to 7 males and 4 females, ranging in age from 18 to 62, with an average age of 34.6 years. The normal cholesterol concentration ranged from 5.35 to 8.20 Gm. per hundred grams of tissue, and the total cholesterol content from 485 to 710 mg. There was no apparent correlation between age and adrenal weight, cholesterol concentration or total cholesterol content. The combined weight of the adrenal glands was greater in males than in females, but the cholesterol concentration tended to be higher in females, resulting in a fairly equal total cholesterol content.

The cholesterol concentration and content of adrenal glands obtained in cases of various types of cardiovascular and/or renal disease and Cushing's syndrome

TABLE 1.—*Normal Persons Who Suffered Sudden Death*

Patient	Age, Yr.	Combined Adrenal Weight, Gm.	Cholesterol Concentration, Gm. per 100 Gm. of Tissue	Total Cholesterol, Mg.
	Males			
H. W.	18	10.54	5.35	564
W. D.	24	9.44	7.28	687
E. S.	25	8.78	6.20	544
J. T.	26	9.18	5.75	528
J. W.	28	8.98	5.58	501
H. G.	57	9.16	6.81	624
C. M.	62	9.45	5.55	524
Average	35.7	9.37	6.07	567
	Females			
A. R.	24	8.20	6.66	546
M. R.	30	8.00	8.02	642
H. C.	35	8.64	5.62	485
M. F.	42	8.78	8.20	720
Average	32.7	8.40	7.12	598

are shown in figure 1. The cases are further divided into those in which death was sudden and those in which death was prolonged. In all cases in which death was considered as sudden, the survival time was a matter of minutes, the longest interval being forty-five minutes.

It is evident from figure 1 that in patients with hypertensive arteriosclerotic heart disease, with or without congestive heart failure, a significant number had cholesterol concentrations and total cholesterol contents which were higher than normal. Abnormal values were also found in hypertensive heart disease in regard to the total cholesterol content. In cases of arteriosclerotic heart disease without failure sudden death was associated with slightly high values, whereas low values were found after prolonged illness. Patients with rheumatic heart disease and congestive heart failure had low cholesterol values. Cases of nephritis are divided into those with and those without hypertension. Nephritic patients with hypertension had higher levels of cholesterol. Regardless of the disease entity, patients who died suddenly had higher adrenal cholesterol concentrations and total cholesterol contents than those who suffered a prolonged death. The influence of age on adrenal cholesterol did not appear to be significant, as it is noted (fig. 1) that

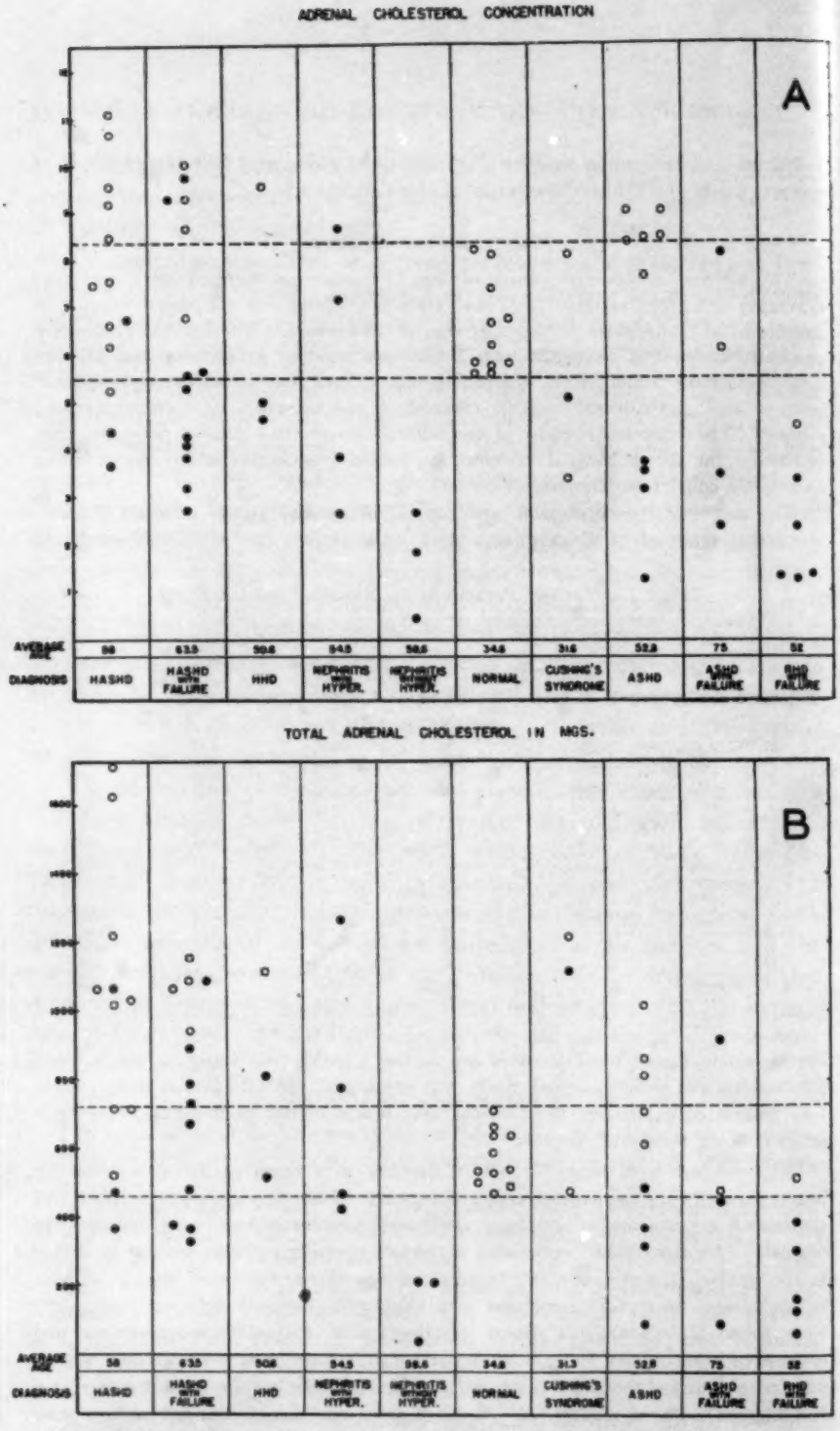

Fig. 1.—Cholesterol of human adrenal glands. *A*, cholesterol concentration in grams per hundred grams of tissue. Open circles denote persons who died suddenly; solid circles, those whose deaths were prolonged. *HASHD* signifies hypertensive arteriosclerotic heart disease; *HHD*, hypertensive heart disease; *Hyper.*, hypertension; *ASHD*, arteriosclerotic heart disease; *RHD*, rheumatic heart disease.

B, total adrenal cholesterol content in milligrams. The symbols and abbreviations are explained in the legend for *A*.

most groups had comparable average ages and yet distinct group variations in respect to adrenal cholesterol existed.

In the group of patients with endocrinopathies, table 2, there were 3 patients who had Cushing's syndrome. Each of the patients had classic symptoms and signs of this disorder, with confirmatory laboratory data. One patient, E. M., a 20 year old unmarried woman, had had symptoms of the disease for one year. Exploration of the adrenal glands revealed bilateral diffuse hyperplasia, and partial adrenalectomy was performed. The portion of the adrenal glands removed had a cholesterol concentration of 3.32 Gm. per hundred grams of adrenal tissue. The total weight of both adrenal glands was postulated to be at least 14 Gm. because of the fact that approximately 90 per cent of one adrenal gland was removed, the weight of this portion being 7 Gm., and both adrenal glands appeared to be of equal size at operation. A 38 year old man who had suffered from Cushing's disease for three and a half years died of acute intestinal obstruction of two days'

TABLE 2.—*Weight of Adrenal Glands and Cholesterol Levels of Patients Who Had Endocrinopathies*

Patient	Diagnosis	Age, Yr.	Sex	Combined Adrenal Weight, Gm.	Cholesterol Concentration, Gm. per 100 Gm. of Tissue	Total Cholesterol, Mg.
E. M.	Cushing's disease	20	F	14.00*	3.32	465
A. G.	Cushing's disease	38	M	22.01	5.08	1,118
H. L.	Cushing's disease	36	F	15.02	8.08	1,206
B. M.	Adrenogenital syndrome	12	F	†	1.92	...
M. J.	Addison's disease	42	F	30.00‡	0.62	186
R. M.	Anorexia nervosa	18	M	14.21	0.25	35
R. B.	Thyrotoxicosis	35	F	10.54	0.43	45
A. L.	Thyrotoxicosis	57	M	10.02	4.03	403
L. L.	Myxedema	59	F	9.45	1.50	142

* See text for derivation of this figure.
† The analysis was made from 90 per cent of the left adrenal gland which was removed at operation.
‡ This patient had Addison's disease on the basis of amyloidosis, which explains the abnormally heavy weight of the adrenal glands.

duration. At autopsy, the adrenal glands were bilaterally enlarged and weighed 22.01 Gm. The cholesterol concentration was 5.08 Gm. per hundred grams of tissue, resulting in a total cholesterol content of 1,118 mg. The third patient was a 36 year old woman who had had signs and symptoms of Cushing's disease for about five years. While in the hospital she suddenly became irrational and comatose and died of a pontile hemorrhage. Her adrenal glands were bilaterally enlarged, the combined weight being 15.02 Gm. The total cholesterol content was 1,206 mg., and the cholesterol concentration was 8.03 Gm. per hundred grams of tissue.

Another patient with hyperadrenocorticism (a 12 year old girl with the adrenogenital syndrome) had 90 per cent of her left adrenal gland removed at operation. The cholesterol concentration of this tissue was 1.92 Gm. per hundred grams of tissue. Previous laparotomy had established the fact that both glands were the site of hyperplasia and that no ovarian or adrenal tumor was present.

One patient with Addison's disease, caused by amyloidosis, had low adrenal cholesterol values. In a case in which an 18 year old sailor had anorexia nervosa, the adrenal cholesterol value was markedly diminished despite the increased

size of the adrenal glands. In 2 cases of thyrotoxicosis the cholesterol values were moderately depressed in one and markedly so in the other.

There were 8 cases of diabetes mellitus in this series. However, all cases were complicated by acute and chronic infections or heart disease, so that the values obtained were not suitable for analysis.

The adrenal cholesterol values of patients with infections were low. In acute infections the average cholesterol concentration was 1.97 mg. per hundred grams of tissue and the average total cholesterol content was 266 mg., the lowest values being noted in cases of meningococcic meningitis and pneumococcic pneumonia. In chronic infections the average cholesterol concentration was 4.44 Gm. per hundred grams of tissue with an average total cholesterol content of 447 mg.

In patients with cancers both the cholesterol concentration and the total cholesterol content were low, averaging 2.09 Gm. per hundred grams of tissue and 311 mg., respectively.

TABLE 3.—*Weight of Adrenal and Cholesterol Levels of Patients Who Suffered from Miscellaneous Diseases*

Diagnosis	Age	Sex	Tempo of Death	Combined Adrenal Weight, Gm.	Cholesterol Concentration, Gm. per 100 Gm. of Tissue	Total Cholesterol, Mg.
Periarteritis nodosa	59	F	Prolonged	9.09	3.83	371
Disseminated lupus erythematosus	26	F	Prolonged	11.79	0.28	33
Alcoholic cirrhosis	53	M	Prolonged	12.05	6.27	755
Alcoholism *	29	F	Sudden	11.10	1.84	204
Alcoholism	31	M	?	10.41	6.33	659
Methyl alcoholism poisoning	30	F	Prolonged	8.47	5.05	427
Avitaminosis	22	F	Prolonged	9.60	3.13	300
Hemochromatosis	41	M	Prolonged	9.40	0.82	77
Hemochromatosis	47	M	Prolonged	....	1.74	...
Pregnancy †	47	F	Sudden	14.53	4.68	673
Duodenal ulcer ‡	60	M	Sudden	11.55	7.45	860

* The patient died of a fractured skull.
† The patient's death was due to an anesthetic accident; abscess of a lung was found at necropsy.
‡ The patient died of pulmonary embolism.

In table 3 pertinent data are given concerning a variety of diseases which were observed. The diverse nature of the cases makes analysis of the results impracticable.

HISTOLOGIC OBSERVATIONS

The normal adrenal cortex contains three distinct zones, namely, the zonae glomerulosa, fasciculata and reticularis. The cells of the normal zona glomerulosa and zona fasciculata have a fluffy and vacuolated appearance, while the cells of the zona reticularis are more acidophilic and frequently contain a yellow-brown pigment. Individual cells (fig. 2 *A*) of the zonae glomerulosa and fasciculata have round nuclei that are fairly light staining, with a moderate number of evenly distributed dark granules. Radiating from the nuclei to somewhat ill defined cell membranes are fine lacelike strands of acidophilic material that enclose small vacuolated spaces, which contain lipid substances.

Whenever the body is subjected to noxious agents, a marked alteration occurs in the cells of the zona glomerulosa and the zona fasciculata (fig. 2 *B*). The

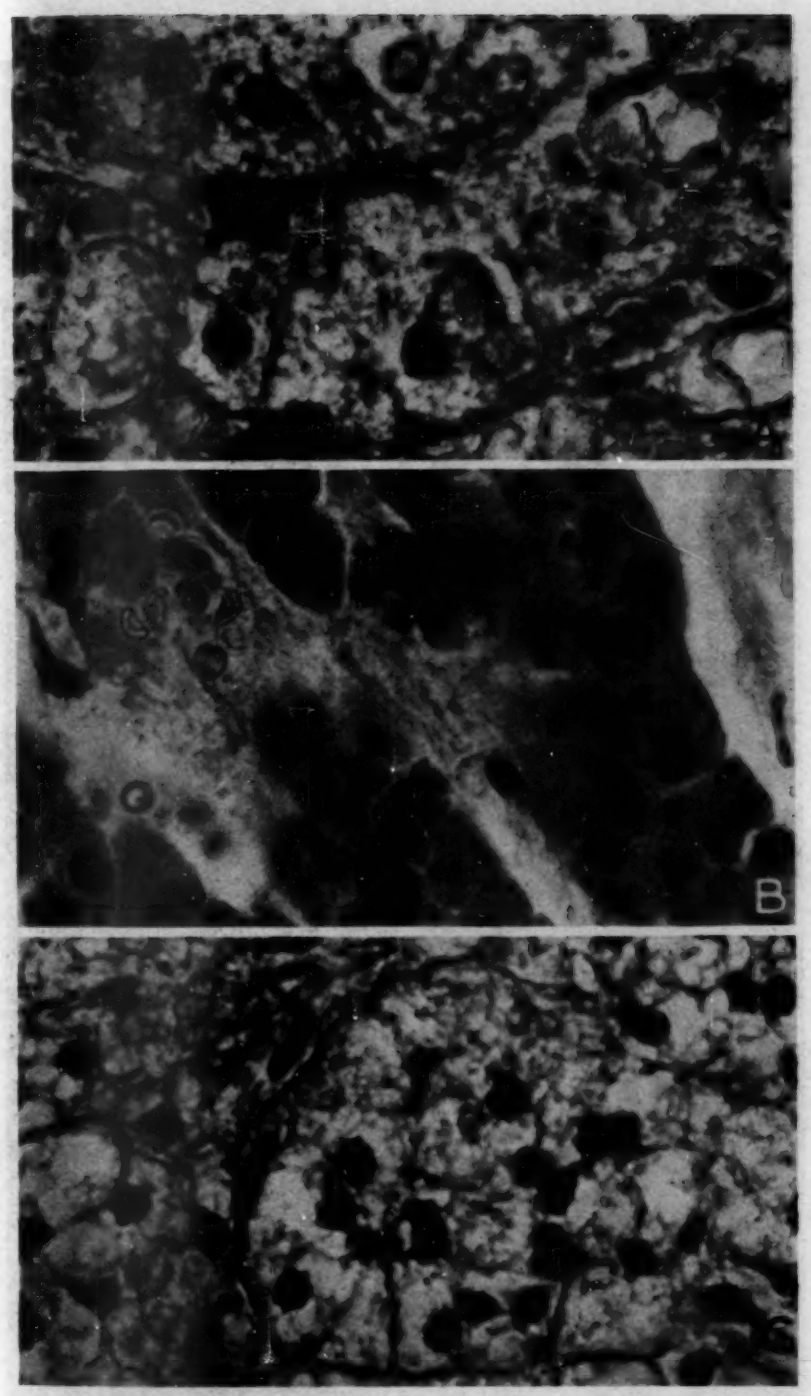

Fig. 2.—*A*, high power view of an outer region of the zona fasciculata of the cortex of a normal adrenal gland. Phloxine–methylene blue; × 1,353.

B, area of the zona fasciculata of the cortex of an adrenal gland from a person who died of an acute hemolytic streptococcus infection of three days' duration. Note the absence of vacuoles and the density of the cytoplasm as compared with the normal zona fasciculata in *A*. Phloxine-methylene blue; × 1,353.

C, histologic change accompanying increase of adrenal cholesterol: type 1. Compare with *A* and note that cell size has increased, vacuoles are larger and nuclei are smaller but that the lacelike cytoplasmic structure has been maintained. Zona fasciculata; phloxine-methylene blue; × 1,353.

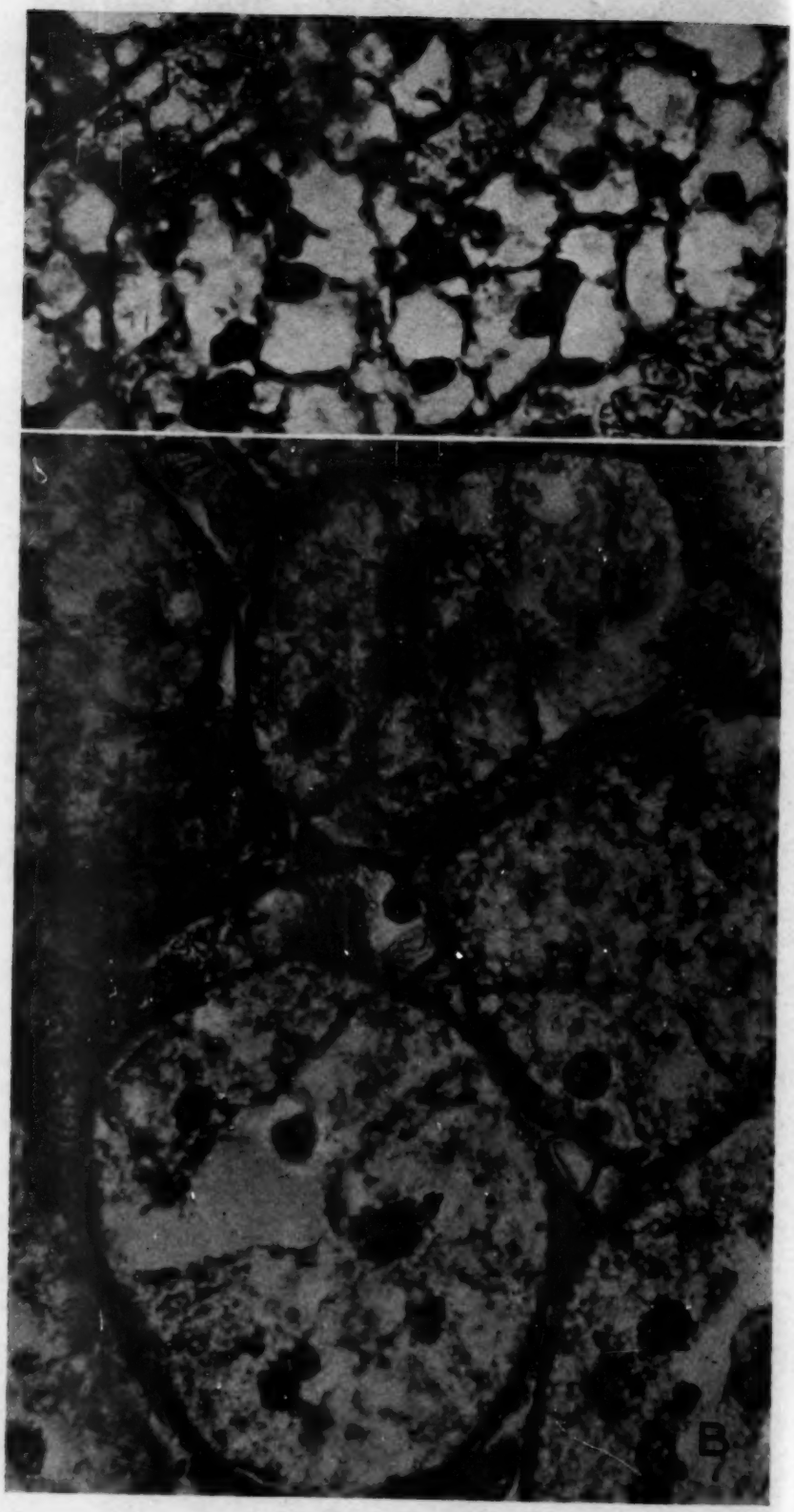

Figure 3

(See legends on opposite page)

vacuoles of these cells disappear, and the cytoplasm becomes diffusely acidophilic. These histologic changes characteristic of lipid depletion are accompanied by a fall of cholesterol and are quantitatively well correlated with the latter.

Adrenal glands that have abnormally high levels of cholesterol also have characteristic histologic appearances that reflect the change of lipid content. We have divided these morphologic alterations into three essential types.

Type 1 is a slight alteration of the normal appearance (fig. 2 *C*), in which the cells are somewhat larger in circumference and the vacuoles appear larger but the cytoplasm retains its delicate framework of acidophilic strands. The nuclei of these cells tend to be smaller and more pyknotic than those of normal cells, and the cell membranes are less distinct.

Type 2 (fig. 3 *A*) is characterized by cells that have small hyperchromatic nuclei that are frequently forced close together by large, round, clear vacuolated spaces filled with lipid substances. In contrast to type 1 the delicate strands of acidophilic framework are sparse, do not stretch across the large clear spaces and are compressed to form the boundaries of these vacuoles.

Type 3 is characterized by small conglomerations of adrenal cortical cells, each group being separated from the others by distinct, moderately thick strands of acidophilic material (fig. 3 *B*). Individual conglomerations contain a varying number (usually 5 to 10) of hyperchromatic, frequently distorted nuclei. Single cell membranes may or may not be evident, but lacelike acidophilic cytoplasmic strands are prominent, producing a fine spongy, vacuolated, appearance. The change in cell structure described as type 3 is the same as has been described in focal and nodular hyperplasia and small adrenal adenomas.

Although three distinct histologic appearances are described, one, two or all three types of change may be observed in a single adrenal cortex, accompanied by transitions between types. In cases of cardiovascular and/or renal disease all three types of alteration were frequently noted (56 per cent of the cases) and when present in a moderate or marked degree adequately accounted for the increased cholesterol values. However, these changes are not specific, for they were found, to a minimal degree, in 18 per cent of the patients dying of other causes.

The degree to which abnormal adrenocortical cells respond to alarming stimuli is of considerable interest, particularly in patients with cardiovascular and/or renal disease. In figure 1 it is apparent that patients of this type whose death was prolonged had lower adrenal cholesterol values than those whose death was sudden, suggesting that alarm produced an adrenal response similar to normal. However, the histologic appearance indicates a response which differs from normal in that the cells which were responsible for the abnormally high levels of cholesterol did not show evidence of depletion of lipid and development of diffusely acidophilic cytoplasm. The accompanying photomicrographs serve to illustrate this point.

The response of type 1 cells is represented in the adrenal glands from a 21 year old woman who died of uremia and chronic pyelonephritis. She had moderate

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Fig. 3.—Histologic change accompanying increase of adrenal cholesterol: *A*, type 2. Compare with figure 2 *A* and note the small, hyperchromatic nuclei and the large, completely vacuolated spaces. Zona fasciculata; phloxine–methylene blue; × 1,211.5.

B, type 3. Note the small conglomerations of cells separated by thick strands of acidophilic staining material. The cytoplasm retains a lacelike appearance. Phloxine–methylene blue; × 1,211.5.

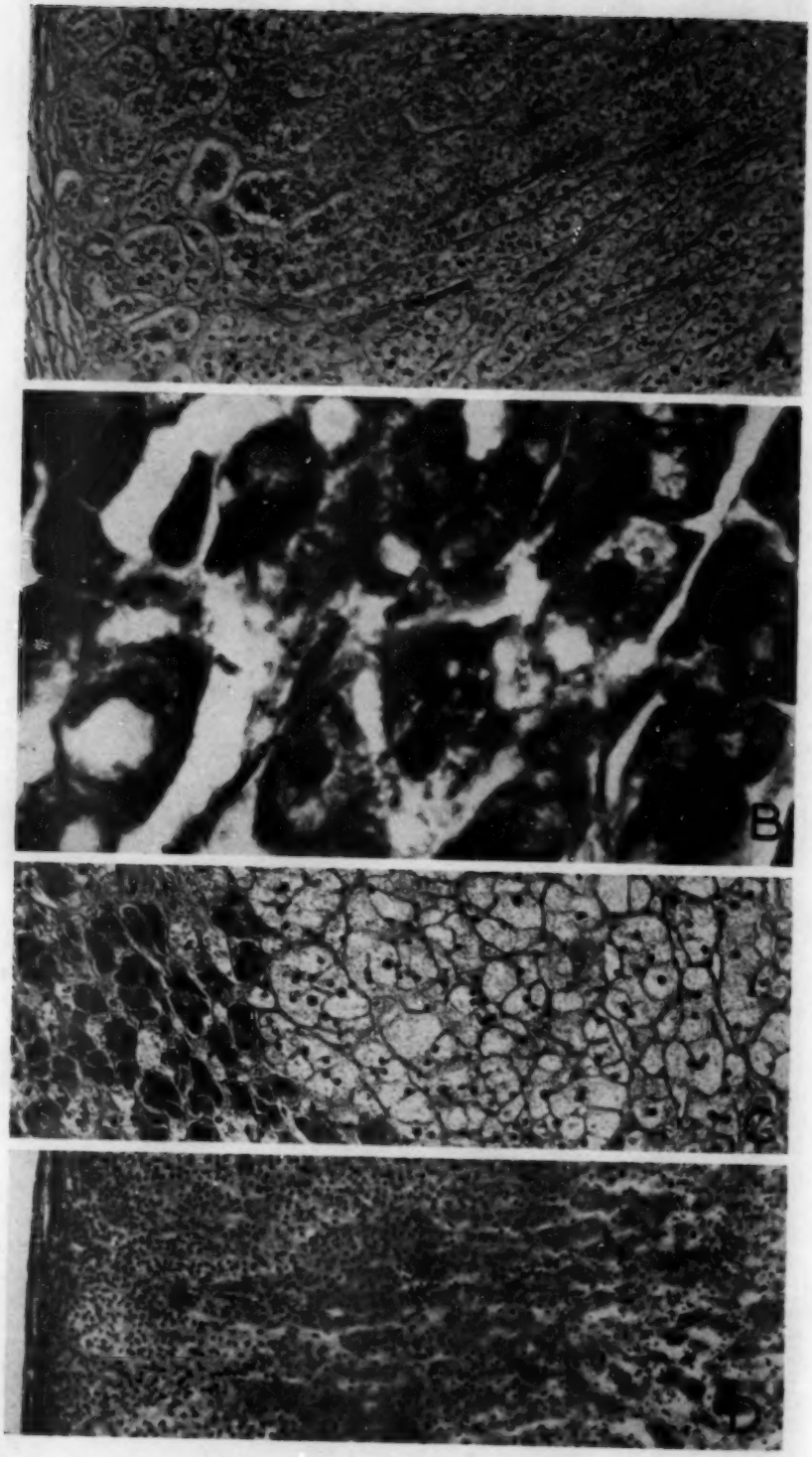

Figure 4

(See legends on opposite page)

hypertension and marked anasarca. During her last eight days of life she had severe potassium intoxication. At autopsy her adrenal glands weighed 14.97 Gm. The adrenal cholesterol concentration was 8.51 Gm. per hundred grams of tissue, and the total cholesterol content was 1,274 mg. Histologic examination revealed that the adrenal cortex was composed almost completely of cells that have been described as those of type 1 (fig. 4 *A*). Only an occasional cell showed evidence of having responded to stress, the rest being completely filled with lipid, despite her severe illness.

Figure 4 *B* shows a section of an adrenal gland from a 60 year old man who had hypertension and an acute myocardial infarction. He lived for four days after the onset of his symptoms of coronary occlusion, during which time he was severely ill, as manifested by shock, cyanosis, dyspnea and other signs of heart failure. Although the cells of the adrenal cortex showed signs of depletion, large clear vacuoles were still evident and apparently had not taken part in the gland's discharge of lipid substances.

Finally, in focal and nodular hyperplasia and in adrenal adenomas, evidence of lipid depletion is most always lacking, although in a few cases a small number of depleted cells were sometimes seen. An example of this lack of response is shown in figure 4 *C*, which illustrates a section of an adrenal gland from a 56 year old man who died of arteriosclerotic heart disease and congestive heart failure. There is no evidence of depletion of lipid in the area of focal hyperplasia (type 3 cells), while in the area of normal structure the cells show diffusely acidophilic cytoplasm.

The presence of the lesions just described, some of which have been called "adrenal hyperplasia," results in an adrenal gland that has a varied histologic pattern from one area to another. In contrast was the adrenal hyperplasia noted in cases of clinical hyperadrenocorticism that consisted of regular and marked cellular proliferation (fig. 4 *D*). Not only was the structure uniform, but the absence of cells characteristic of a high lipid content was notable. This would be expected in patients E. M. and B. M. (table 2), both of whom had low cholesterol values. In patients H. L. and A. G., who had higher cholesterol values, cells of types 1 and 2 were seen, but their extent was minimal and overshadowed by the predominant abnormality of diffuse hyperplasia consisting of cells of normal or low lipid content.

COMMENT

There are few figures in the literature with which to compare our normal values of adrenal cholesterol concentration (5.35 to 8.20 Gm. per hundred grams of tissue) and total adrenal cholesterol content (485 to

Fig. 4.—*A*, adrenal cortex from a 21 year old woman with uremia and potassium intoxication. Despite her severe illness, note the apparent lack of adrenal response as evidenced by lipid-filled cells. Both adrenal cortices had this appearance throughout. Phloxine–methylene blue; × 184.

B, zona fasciculata of an adrenal cortex showing evidence of depletion. However, the large clear vacuoles remain and show no evidence of depletion. Compare with figure 3 *A*. Phloxine–methylene blue; × 1,289.

C, section of an adrenal cortex from a person who died of arteriosclerotic heart disease and congestive heart failure. Note the depletion of cells in an area of normal structure at the left, but the absence of such a change in an area of focal hyperplasia at the right. Phloxine–methylene blue; × 230.

D, adrenal cortex of E. M., table 3, with Cushing's syndrome. Note the regular and extensive hyperplasia and the absence of cells with a high lipid content. Phloxine–methylene blue; × 115.

720 mg.). In 1935 Ewert[8] published values of the adrenal cholesterol of 11 persons whom he considered to be normal. Six cases were collected from the literature, and several criticisms may be made. For instance, 2 of these cases were taken from Wacher and Hueck[9]; one person was killed in a railroad accident, while the other died of lysol® poisoning. The adrenal cholesterol concentration in these persons was 1.6 and 2.4 Gm. per hundred grams of tissue, respectively. However, the authors did not say whether death was sudden or not, or whether the rest of the organs were without evidence of disease at autopsy. Ewert quoted Fex,[1c] who reported "normal" adrenal cholesterol values of 4 persons. Of these 4 persons, only 1 may be considered normal, a 30 year old woman who died suddenly of suffocation and whose adrenal cholesterol concentration was 4.0 Gm. per hundred grams of tissue. The remaining patients died from one to four days after their fatal accident, which was adequate time for an "alarm reaction" to take effect on the adrenal cholesterol. Ewert's own normal subjects appear to have been persons who died suddenly. He reports values ranging from 2.8 to 5.1 Gm., with an average of 3.9 Gm., per hundred grams of tissue. He used a gravimetric procedure to determine the cholesterol content and commented that such a procedure gives values approximately 30 per cent lower than colorimetric methods.

Low cholesterol values are found in the adrenal glands of persons dying of such conditions as acute infections, burns and trauma. Living persons subjected to these same types of stress have shown, by both biologic and chemical methods, increased excretion of "cortin-like" compounds[10] and of 17-ketosteroids—the latter transiently rising for from one to three days, followed by a period in which the excretion is usually below normal.[11]

In chronic diseases the adrenal cholesterol values are moderately depressed. There are too few estimations of the "cortin" excreted in comparable living persons to draw conclusions, but from isolated reports it appears that the levels may be low, normal or increased. However, the amount of 17-ketosteroids excreted by chronically ill and debilitated persons is usually below normal.[11] Talbot and co-workers[5a] and Venning and Browne[5b] pointed out that glycogenic corticoid compounds

8. Ewert, B.: Upsala läkaref. förh. **40**:421, 1935.

9. Wacher, L., and Hueck, W.: Arch. f. exper. Path. u. Pharmakol. **71**:373, 1913.

10. Weil, P., and Browne, J. S. L.: J. Clin. Investigation **19**:772, 1940. Shipley, R. A.; Dorfman, R. I., and Howitt, B. N.: Am. J. Physiol. **139**:742, 1943. Venning, E. H.; Hoffman, M. M., and Browne, J. S. L.: J. Biol. Chem. **148**:455, 1943. Talbot, Albright, Saltzman, Zygmuntowicz and Wixom.[5a] Venning and Browne.[5b] Shipley, Dorfman, Buchwald and Ross.[5d]

11. Forbes, A. P.; Donaldson, E. C.; Reifenstein, E. C., Jr., and Albright, F.: J. Clin. Endocrinol. **7**:264, 1947.

and 17-ketosteroids frequently do not parallel each other. When glycogenic corticoid compounds increase, the excretion of 17-ketosteroids tends to be depressed. Selye [12] attributed this phenomenon to a "hormonal shift" in which during times of stress the adrenal gland increases its production of corticoid compounds but decreases its production of testoid compounds.

Thus, the evidence so far accumulated strongly points to the fact that under stress, particularly that of an acute nature, the cholesterol content of the human adrenal gland decreases and that this change is accompanied by an increased excretion of "cortins."

On the other hand, it cannot be said that whenever the adrenal cholesterol is low the excretion of "cortins" is high. This is borne out by the fact that in Addison's disease, panhypopituitarism, anorexia nervosa and certain chronic debilitating diseases in their terminal states, the adrenal cholesterol concentration is low, as is the excretion of "cortins."

Cases of hyperadrenocorticism afford the best examples for study of adrenal cholesterol where adrenal hyperfunction is of a sustained nature. We did not find the adrenal cholesterol concentration above normal in any case. It was very low in the patient with the adrenogenital syndrome and moderately depressed in 1 patient with Cushing's syndrome. Although the adrenal glands of both these patients were removed at operation, and some "alarming" stimuli occurred, it seems likely that the glands were extirpated before the adrenal cholesterol values were affected significantly. The elevation of the total adrenal cholesterol contents of the remaining 2 patients with Cushing's syndrome is attributable to the increased weight of the adrenal glands, not to an abnormally high adrenal cholestrol concentration.

As a decreased concentration of adrenal cholesterol may be associated with an increased or a decreased production of "cortins," and a high concentration of adrenal cholestrol was not present in cases of hyperadrenocorticism, it seems apparent that the rate of hormone production is not dependent on the concentration of adrenal cholesterol.

The clinical observations that Cushing's syndrome is usually associated with hypertension, and Addison's disease with hypotension, and that desoxycorticosterone may produce hypertension in patients with Addison's disease [13] and an increase of blood pressure in normal persons [14] arouse interest in the relation of function of the adrenal gland to hyper-

12. Selye, H.: J. Clin. Endocrinol. **6**:117, 1946.

13. Thorn, G. W.; Dorrance, S. S., and Day, E.: Ann. Int. Med. **16**:1053, 1942. Ferrebee, J. W.; Ragan, C.; Atchley, D. W., and Loeb, R. F.: J. A. M. A. **113**:1725, 1939.

14. Perera, G. A.; Knowlton, A. I.; Lowell, A., and Loeb, R. F.: J. A. M. A. **125**:1030, 1944.

tension. This interest is furthered by the results of animal experiments. Selye [12] has produced vascular lesions similar to those seen in hypertension and nephrosclerosis by the administration of desoxycorticosterone and crude anterior pituitary lobe extracts. Investigators [15] have found that adrenalectomy reduces an animal's response to renin and that the adrenal cortex is necessary to maintain hypertension in Goldblatt animals. Thus, the finding of increased amounts of cholesterol in some adrenal glands from people who die of cardiovascular and/or renal disease, coupled with the fact that there is a decrease of adrenal cholesterol associated with elaboration of adrenal cortical hormones, poses the problem of the significance of the elevated adrenal cholesterol values in relation to hypertension.

Do these high cholestrol values mean continual increased production of adrenal hormones, or potentially increased production of hormones, or are they merely the reflection of an adrenal gland which has undergone repeated cycles of stimulation, depletion and "involution"? We believe that the last explanation is the most likely, although it is impossible to exclude the first two possibilities.

In regard to continual increased production of hormones it seems probable that if the function of adrenal cortex is an etiologic factor of hypertension, the electrolyte-controlling hormones of the desoxycorticosterone type play the most important role. Unfortunately, there is at present no satisfactory method of assaying the excretion of the hormones or of their products which influence electrolyte metabolism. Thus, important as they are, determinations of the output of this type of adrenal hormone in normal and in hypertensive persons are not available.

If the theory that high cholesterol levels signify potentially increased hormone production is true, histologic examination should confirm this theory; that it does not is evidenced by the fact that cells which have accumulated excess amounts of lipid do not usually respond to stress with a depletion of lipid as do normal cells. In addition, it is important to note in regard to potentially increased production of hormones that adrenal hypertrophy and high cholesterol values, although frequently found in cases of hypertension, are by no means consistently present.

One of the most pertinent observations concerning the possibility that increased cholesterol values signify repeated stimulation followed by "involution" is that of Ludewig and Chanutin.[16] These authors followed the adrenal cholesteral and ascorbic acid concentrations for seven days in rats subjected to thermal burns and to parenteral injection and

15. Friedman, B.; Somkin, E., and Oppenheimer, E. T.: Am. J. Physiol. **128**:481, 1940. Williams, J. R.; Diaz, J. T.; Burch, J. C., and Harrison, T. R.: Am. J. M. Sc. **198**:212, 1939. Houssay, B. A., and Dexter, L.: Ann. Int. Med. **17**:451, 1942. Goldblatt, H.: ibid. **11**:69, 1937.

16. Ludewig, S., and Chanutin, A.: Endocrinology **41**:135, 1947.

cutaneous application of nitrogen and sulfur mustards. They noted that after an initial depression the cholesterol regularly rose to supernormal levels, which were still present at the end of seven days. It should also be noted that prolonged administration of an anterior pituitary extract containing the adrenocorticotropic factor [2b] or of crude pituitary extracts [17] causes an increase of lipid in the adrenal gland, and presumably there was initially depletion of lipid.

Experiments that show marked and rapid fluctuations of adrenal cholesterol, the initial response being depletion, which is later followed by abnormally high levels of cholesterol, form the basis for an explanation of an abnormally high concentration of adrenal cholesterol and the histologic changes that reflect this high concentration.

It seems logical that as the adrenal gland is a dynamic organ its deviations from normal structure and chemical constitution are reflections of periodic activity and "involution." Although "involution" is a term not usually applied to the adrenal cortex, it seems particularly fitting. By analogy one might consider the thyroid gland, in regard to which the term "involution" has had widespread use. For example, when that gland is called on to maintain the homeostatic equilibrium of the body—for instance, in such situations as iodine lack and pregnancy—there follows a series of histologic changes in which hypertrophy, hyperplasia and involution occur. Although during and after this sequence of events there may be no evidence of hyperfunction or hypofunction, the end result is frequently an enlarged gland which is the site of varying histologic patterns and adenomas.

The concept that excess deposits of lipid substances, focal and nodular hyperplasia and small adenomas of the adrenal cortex are the result of cycles of stimulation, depletion and involution may well explain the common occurrence of such lesions in the wide variety and apparently unrelated circumstances in which they are found.[18]

Our present interpretation of the abnormally high levels of cholesterol found in some cases of cardiovascular and/or renal disease, particularly those with hypertension, is that the adrenal glands of the patients have been the site of repeated cycles of stimulation and "involution," the end result being morphologic alterations and the storage of excessive amounts of cholesterol. This implies that over a long period of time secretion of adrenal cortical hormones may have been excessive, but it does not imply that adrenal glands containing high levels of cholesterol or adenomas are the site of continual excessive production of cortical hormones such as is believed to occur in Cushing's syndrome.

17. Emery, F. E., and Atwell, W. J.: Anat. Rec. **58**:17, 1933-34.

18. Moore, R. A.: A Textbook of Pathology, Philadelphia, W. B. Saunders Company, 1944, pp. 1068-1069. Goldzieher, M.: The Ardenal Glands in Health and Disease, Philadelphia, F. A. Davis Company, 1944, pp. 97-103. Grollman, A.: The Adrenals, Baltimore, Williams & Wilkins Company, 1936, p. 337.

SUMMARY AND CONCLUSIONS

The cholesterol contents of 125 pairs of adrenal glands were determined. It was found that when rigid criteria of normality were adopted in selecting material for study, namely, the adrenal glands of persons who suffered sudden death and who at autopsy showed no pathologic changes other than those caused by the fatal agent, the cholesterol values were relatively constant. In 11 normal persons the adrenal cholesterol concentration ranged from 5.35 to 8.20 Gm. per hundred grams of adrenal tissue and the total adrenal cholestrol content from 485 to 720 mg.

Cholesterol values were also determined on adrenal glands from persons who died of cardiovascular and/or renal disease (some having undergone sudden and some prolonged deaths) and from persons who succumbed to infections, cancer and hepatic or endocrine diseases. Included in the endocrine group were 3 patients who had Cushing's syndrome and 1 patient who had the adrenogenital syndrome.

Abnormally high adrenal cholesterol levels were frequently found in persons who died of cardiovascular and/or renal disease. In 4 persons with hyperadrenocorticism the adrenal cholesterol concentration was either low or within normal limits. Persons with infectious diseases and chronic debilitating diseases had low cholesterol values.

In adrenal glands which contained abnormally large amounts of cholesterol three types of cellular change were observed that were the histologic manifestations of increased cholesterol content. It was noted that when an adrenal gland which revealed any one or all of these changes was the site of increased physiologic activity, these abnormal cells did not show depletion of lipid as normal cells do.

An attempt to correlate adrenal cholesterol values with the available data on the excretion of "cortin-like" substances and 17-ketosteroids revealed that when the body has undergone "stress" the excretion of adrenal steroids is high and the adrenal cholesterol content is low. However, low adrenal cholesterol values are not always associated with increased excretion of adrenal steroids, and in hyperadrenocorticism, in which there is sustained excessive production of adrenal hormones, the cholesterol concentration was found to be normal or low. Thus, it would seem that the rate of adrenal hormone production is not necessarily dependent on, or reflected by, the adrenal concentration of cholesterol.

The significance of increases of adrenal cholestrol and the mechanism by which they occur are discussed.

It is suggested that high levels of adrenal cholesterol and their histologic manifestations are the result of repeated cycles of adrenal stimulation and depletion followed by "involution" and storage of excessive amounts of cholesterol.

RETICULUM CELL SARCOMA OF BONE

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THE MAJORITY of primary cancers of bone present an almost hopeless prognosis. Heroic surgical measures, even if started in the early stages, are often unsuccessful in preventing a rapid and fatal termination in osteogenic sarcoma.

Parker and Jackson [1] have, however, isolated a group of primary tumors of bone which present a more hopeful outlook, especially if the conditions are recognized early and treated properly. As a result of a study of 3 tumors of this group of their own and the material from the Registry of Bone Sarcoma of the American College of Surgeons, they were able to report on 17 tumors which formerly "had been variously classified as Ewing's sarcoma, Hodgkin's disease, lymphosarcoma, osteogenic sarcoma, leucosarcoma or as inflammation." They defined the clinical, roentgenologic and histologic characteristics of this group. The Registry of Bone Sarcoma recognized these tumors as a separate entity in 1939.

These neoplasms, in spite of their clearcut characteristics, have not been sufficiently recognized by clinicians, radiologists and pathologists. "The accurate diagnosis of a bone sarcoma remains a rather difficult task. Unless the surgeon and pathologist are familiar with what may happen in the bone, he is hardly able to recognize what has happened." [2] It is therefore believed that a report of 5 cases observed during the last six years might help to draw greater attention to this interesting group of bone tumors.

REPORT OF CASES

CASE 1.—An emaciated Deccani Hindu, 30 years old, was admitted to Tata Memorial Hospital because of pain in the right thigh. One year before, he had had shooting pains in the hip, which were worse at night. His physician thought that he had tuberculosis of the joint and put the limb in a plaster cast for two months. The treatment did not relieve his pain. His condition became worse. He lost much weight and was unable to walk.

On his entry it was found that the right leg was fixed and incapable of either active or passive movements. Resistance to deep palpation was encountered in the right inguinal region and over the corresponding trochanteric area. A fulness

1. Parker, F., and Jackson, H.: Surg., Gynec. & Obst. **68**:45, 1939.
2. Ewing, J.: Surg., Gynec. & Obst. **68**:971, 1939.

was felt in the right lower quadrant of the abdomen as if arising from the pelvic surface of the pubic bone. There was a tenderness on the right side on rectal examination. Roentgen examination (fig. 1) showed a generalized rarefaction of the upper third of the right femur. There was no marginal sclerosis. Similar areas of bone destruction were seen near the pelvic border of the acetabulum, the lower part of the ilium and the ischium on the right side. There was a feeble attempt at a periosteal reaction, as evidenced by the laying down of parallel lamellas of bone in the subtrochanteric portion of the femur. Below the main lesion of the thigh bone the cortex was denser than normal. There was almost complete absence of bone trabeculae in the medullary portion in this region. The dorsal portions of the fourth and fifth ribs on the left side showed similar areas of bone destruction. The blood did not show any significant change from the normal except for mild secondary anemia.

Fig. 1 (case 1).—Roentgenogram of the femur and the right side of the pelvis showing changes in the ilium, the ischium and the upper third of the femur.

Fig. 2 (case 1).—Photomicrograph of tissue from the fifth left rib, showing reticulum cell sarcoma. Hematoxylin and eosin stain.

These was no Bence Jones protein in the urine.

About 4 cm. of the diseased portion of the fifth left rib was resected. The rib was adherent to the pleura posteriorly, and a portion of its wall had been destroyed by disease. The bony tissue was replaced by a grayish soft granular opaque material with irregular rough spicules projecting through it. The histologic examination (fig. 2) revealed the structure of reticulum cell sarcoma. (The microscopic characters of the tissue in all the 5 cases were so similar that they have been described later in one place, to avoid repetition.)

The patient was treated with roentgen radiation. His general condition remained good, and toward the end of the treatment he was able to move the thigh through 5 degrees. The pain in the thigh and the chest disappeared, and he was able to walk about on crutches.

During the first course of radiation a lesion developed in the right scapula, which on roentgen examination showed an elliptic area of rarefaction along the axillary border. After the completion of the course of radiation a piece of the fourth rib was resected from the region previously diseased. On microscopic

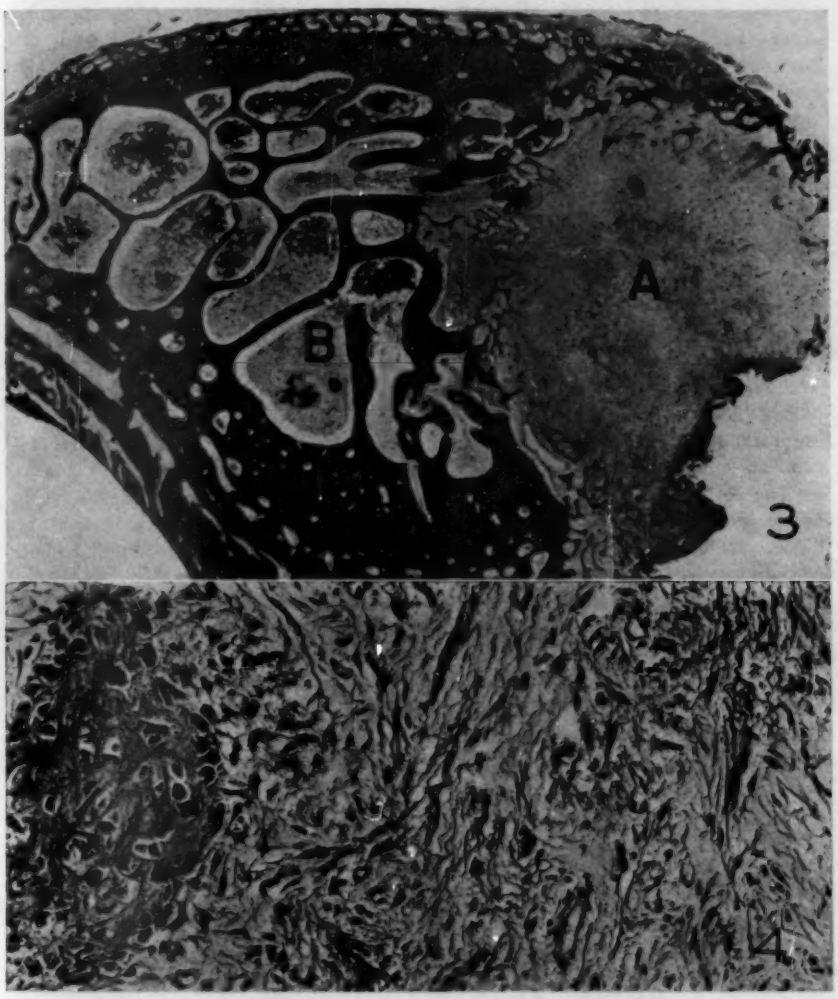

Fig. 3 (case 1).—Transverse section of a rib showing an area in which the bone had been destroyed by sarcoma replaced by fibrovascular connective tissue, marked *A*, and dark staining areas in which fragments of dead bone are seen lying in the marrow, marked *B*. Hematoxylin and eosin stain.

Fig. 4 (case 1).—A higher magnification of the region marked *A* in figure 3 to show the microscopic features of the healed area. Hematoxylin and eosin stain.

examination (fig. 3) it showed not only that the tumor had completely regressed and been replaced by a fibrovascular granulation tissue but also that new trabeculae were being laid down and the bone reproduced. A study of this tissue was

particularly interesting because of the information which it supplied concerning the new bone being formed on the site of a healed tumor.

The patient continued to have some stiffness in the right hip and a little tenderness over the upper part of the right femur for nearly one year. He was gradually able to resume his work and now, three years later, is still alive and well.

CASE 2.—A 20 year old Hindu Deccani farmer was admitted for tumor of the cheeks and inability to swallow solids. He stated that he had noticed painless swelling on the lower jaw some four months previously. It had gradually involved the whole jaw and had spread to both cheeks. Three months later the swelling of the right cheek had burst inside and was discharging foul purulent matter.

There was a large painless swelling occupying almost the whole of the right side of the face. It was firm in front and above, but soft and fluctuating elsewhere. There was a smaller and harder swelling on the opposite side. Inside the mouth, the lower alveolus was thickened by a large elastic nodular growth. The tumor had spread laterally to both cheeks and posteriorly to the anterior pillars of the fauces. It had ulcerated on the right side. The patient was unable to close his mouth. The nodular masses were projecting into the oropharynx and were almost occluding its lumen. There was a hard node in the left submandibular region. The blood showed mild secondary anemia. Blood studies, including determinations of both alkaline and acid phosphatases, gave normal results. A roentgenogram of the mandibles showed multiple rounded areas of rarefaction involving the descending rami on either side of the mandible. A biopsy showed reticulum cell sarcoma.

The patient was started on high voltage roentgen ray therapy and felt so much better after five exposures that he discontinued treatment and went back to his home. He returned after fourteen months. The main bulk of the original tumor had disappeared, but a mass was present over the left maxilla and the parotid gland; there was a swelling also in the region of the tonsils and the soft palate. He was given another course of high voltage roentgen ray therapy. There was rapid regression of the disease. Five months later no disease could be seen on the right side, but there was a brawny swelling in the left preauricular region with softening in the center. This prevented him from opening his mouth or chewing his food. He was further treated with roentgen rays. He was soon able to eat solid food easily, and the pain was relieved. He appeared one month later with a large painful swelling on the left side of his face. The swelling burst through the cheek and discharged large sloughs of necrotic material. Biopsy did not reveal any evidence of neoplastic tissue. The patient's condition rapidly improved, and he left the hospital one week later.

CASE 3.—A 37 year old Rajput cavalryman was referred for a massive swelling of the left shoulder and chest. Six months previously, he had had pain in the left shoulder. Some days later he noticed a swelling in the same region. The swelling gradually increased in size and spread to the armpit and the front of the chest.

He was a well built, athletic man with a large smooth swelling over the upper part of the left side of the chest. There were small firm nodes in the supraclavicular region and a larger mass in the axillary region. The swelling was firm, elastic and slightly painful. There was no fever. A roentgenogram showed that the major portion of the axillary borders of the left scapula was destroyed. There was a huge, soft tissue tumor, which revealed scattered strands of ossification in its substance. The neck of the glenoid process showed condensation of bony trabeculae.

There was slight anemia; otherwise cytologic and chemical analyses showed that the blood was normal. Biopsy specimens taken from the infraclavicular region

showed the characteristics of a reticulum cell sarcoma. The patient was treated with roentgen radiation. The sarcoma began to melt away after three or four treatments, only to appear at some other place on the trunk and the arm. After a couple of months a firm mass developed in the left iliac fossa. The masses regressed with treatment, but new ones cropped up elsewhere. His condition became steadily worse, and because of the low white cell count further irradiation had to be discontinued. The patient died at a military hospital two months after the last radiation treatment. No further information could be obtained.

Case 4.—A strongly built Moslem, 38 years old, was referred because of a large swelling on the chest. His illness had started with severe asthmatic attacks and precordial pain, eighteen months previously. His condition became steadily worse. There never had been any lymphadenopathy. The white blood cell count was 14,800, with 55 per cent eosinophilic granulocytes. He was therefore believed to have been suffering from Löffler's syndrome, and he was given neoarsphenamine intravenously. His asthmatic symptoms improved dramatically; but a lump developed on the sternum. A couple of months later he began to complain of shooting pains starting in the lower dorsal region, passing down his legs and radiating round his trunk to the epigastrium. The pain appeared only when the patient sat up and bent forward, and was aggravated by coughing. He was treated with high voltage roentgen rays.

About halfway through the treatment a roentgenogram showed marked destruction of the body of the eighth thoracic vertebra. The intervertebral disks were intact. There was a similar but less marked involvement of the first lumbar vertebra.

A swelling 7 cm. in diameter projected about 3 cm. beyond the general outline of the chest over the manubrium. There was a firm node in the right axilla, and a larger softer swelling in the left axilla. There was a small discrete node in the supraclavicular region on the right side, which was excised for examination. The right tonsil was swollen and red but not ulcerated. The patient complained of fleeting pains in the arms and the calves. A roentgenogram of the chest revealed a roughly globular expansion of the manubrium and a well defined small area of rarefaction in the lower segment of the sternum. The excised node showed the characters of a reticulum cell sarcoma.

Owing to the unsatisfactory conditions in the city, the patient decided to go back to his home town and have radiation therapy there.

Case 5.—A 22 year old Deccani Hindu farmer, well built but extremely emaciated, was admitted to the hospital with retention of urine and inability to move his legs. His complaint had started only six weeks earlier with swelling of the lower jaw and inability to eat. A fortnight later he began to have lightning-like pains in both legs. The pain was much worse at night. This continued for seven days, and on the eighth, as he could not bear the pain any longer, he branded himself with a hot iron. The pain disappeared, but he was unable to move his legs. He became bedridden, and bed sores developed. A few days later he was unable to urinate.

The gums were soft, swollen and spongy. All the teeth were loose. There were marked wasting and loss of movement in both legs. Sensation was absent up to 5 cm. above the symphysis pubis. The knee and ankle jerks were absent. The condition of the patient steadily deteriorated. A fortnight later he died.

Autopsy (summary).—The gums along their whole length in both the maxilla and the mandible were swollen and soft. The color was reddish yellow. Most

of the bony part of the mandible was replaced by a soft growth, which had eroded the bone unevenly. The teeth were loosely embedded in this spongy mass.

The submental, submaxillary and cervical lymph nodes were all moderately enlarged. The spleen was slightly increased in size.

A yellow mass was infiltrating the bodies of the eleventh and twelfth thoracic and the first lumbar vertebrae and the upper part of the psoas muscle in front.

The tumor of the jaws and the spine revealed the characteristics of a reticulum cell sarcoma. The major portion of the tumor tissue was composed of a delicate network of reticulum, the meshes of which were occupied by large cells with pale nuclei. The tumor was traversed by a rich plexus of capillaries and contained areas of hemorrhage and necrosis. There were few remnants of fine spicules of bone in the tumor tissue. The most advanced lesion was seen in the mandible.

GENERAL CONSIDERATIONS

"The existence of a pure reticulum cell lymphosarcoma of bone marrow was recognized many years ago by Kaufmann. He stated that the cells were larger than plasma cells, resembled large or small reticulum cells, with giant cells, and a reticular matrix was present." [2] However, the recognition of these tumors as a separate clinical entity and a definition of their clinical, roentgenologic and pathologic features is due to Parker and Jackson. The case described by Lefdahl and Levine [3] and case 14 of Craver and Copeland [4] probably belong to this class. Since Parker and Jackson published their observations, 3 further cases [5] have been reported in American literature. The reports published in *Acta Radiologica* [6] were not available. It may now be worth while to review the general features of these tumors on the basis of the reports in the literature and personal observations.

Clinical Aspects.—Primary reticulum cell sarcoma of bone is mainly a disease of younger persons. Parker and Jackson pointed out that whereas in more than three fourths of the cases generalized reticulum cell sarcoma occurs after the age of 40, in about the same proportion of cases reticulum cell sarcoma of bone occurs under that age.

The disease is often ushered in with severe shooting pain referable to the site of the lesion. This was so in 3 of the 5 cases now reported and in 13 of the 17 cases reported by Parker and Jackson. It was also a noticeable feature in the case reported by Edwards and in one of the cases of Szutu and Hsieh.[5b] The disease progresses slowly in most cases and causes marked destruction of bone. It tends to remain localized for a long time, and a large tumor may be accompanied by

3. Lefdahl, G., and Levine, V.: Arch. Path. **21**:869, 1936.

4. Craver, L. F., and Copeland, N. M.: Arch. Surg. **28**:809, 1934.

5. (*a*) Edwards, J. E.: Am. J. Path. **26**:835, 1940. (*b*) Szutu, C., and Hsieh, C. K.: Ann. Surg. **115**:280, 1942.

6. Eker, R., and Poppe, E.: Acta radiol. **23**:387, 1942. Rosendal, T.: ibid. **26**:210, 1945.

few if any metastases. The disease extends into the neighboring muscle and connective tissue and spreads slowly along the lymphatic channels to involve adjacent lymph nodes. There is a predilection for metastases in other bones, sometimes in those of the spine and hardly ever in those of the skull. The most arresting feature is the relative well-being of the patient and his lack of concern about his disease. It was frequently noticed that the attending physician was more alarmed at the extent of the morbid condition than the patient himself. It is necessary to point out that the prognosis of the disease is relatively favorable if it is diagnosed early and prompt measures are adopted toward its cure. The cytologic and biochemical studies in the first 4 cases did not reveal any significant deviations from the normal. In 1 case only the acid phosphatase was higher than normal, but well below the figure in metastatic deposits in cancer of the prostate. The physical signs referable to a disease of bone often raise a clinical suspicion of syphilis, but in the cases of reticulum cell sarcoma the blood is uniformly normal. It is therefore necessary to warn against prolonged anti-syphilitic treatment with a harmful delay of suitable treatment.

Roentgen Diagnosis.—The diagnostic roentgen findings are definite, but not characteristic of the disease. They consist of a mottled or diffuse osteolytic process with little evidence of new bone forming at the periphery of the lesion or under the periosteum. Bone trabeculae appear to melt away as they are involved in the sarcomatous infiltration. The roentgenographic appearances are often confused with those of other tumors or inflammatory lesions of bone. It is likely that with greater appreciation of, and familiarity with, roentgenographic pictures of the disease a correct roentgenologic diagnosis may be expected more frequently.

Structure.—The macroscopic examination of the tumor reveals a grayish soft granular homogeneous tissue dotted with dark reddish areas of hemorrhage and necrosis. There is absence of coarse spicules or trabeculae of bone in most of the tumor mass. The microscopic structure of the tumor is characteristic, as are also the cells which compose it. The tumor tissue is held in a loose fibrovascular framework. The collagen bands are sparse and thin. The intervening space is occupied by a delicate interlacing of argyrophil fibers. These fibers seem to be woven round individual cells or small groups of them. The tumor tissue is richly supplied with blood capillaries. The cells lining the capillaries are often cuboidal, swollen and several layers thick. The tumor cells seem to insinuate themselves between the lining endothelial cells and to push into the capillaries. The vessels present a peculiar petaled appearance in silver preparations. Infarction, degeneration and necrosis occur freely in the tumor. The characteristic cell composing

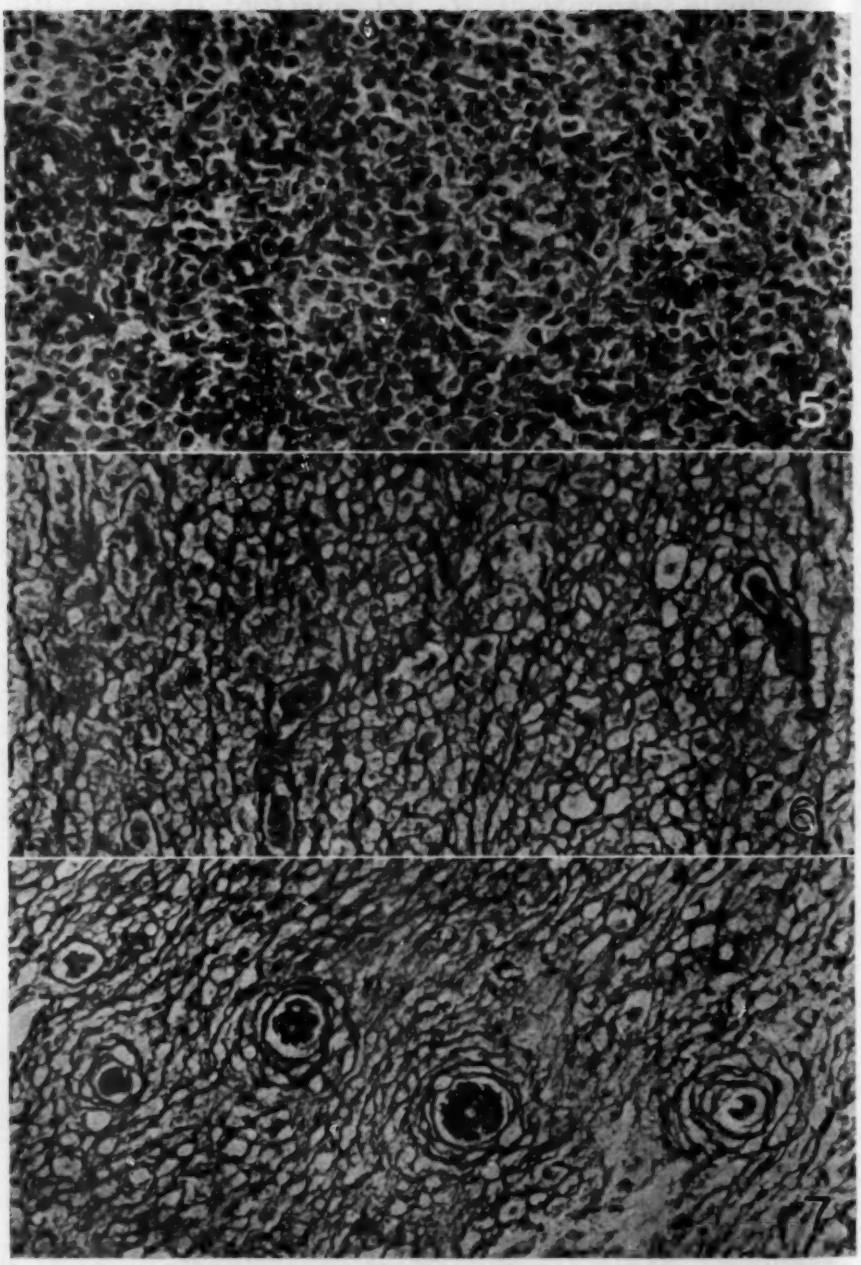

Fig. 5 (case 3).—Photomicrograph of reticulum cell sarcoma. The cells are smaller and more irregular in size and their nuclei are more hyperchromatic than those in case 1. Compare this section with that in figure 2. Hematoxylin and eosin stain.

Fig. 6 (case 1).—Photomicrograph showing the delicate network of reticulin fibers between individual cells or small groups of them. Gömöri reticulum stain.

Fig. 7 (case 1).—High power photomicrograph showing the peculiar petaled appearance of the walls of small blood vessels. Gömöri reticulum stain.

the tumor is a rather large cell, about 20 microns in diameter, with abundant amphophilic or weakly acidophilic homogeneous cytoplasm. The cell borders are vague and irregular. The cells are usually globular, but in some tumors narrow, elongated forms or spheroidal cells with blunt cytoplasmic processes are frequently seen. The nuclei are round, ovoid and occasionally kidney shaped. They are usually large (10 to 12 microns), pale, with distinct nuclear membrane and fine chromatin granules. Some tumors contain cells with a distinct nucleolus. Mitotic figures are infrequent, and atypical mitoses are rare. Large cells or giant cells may occasionally be seen, but they do not resemble Sternberg-Reed cells. In some tumors the cells appear actively phagocytic and contain deeply stained ingested particles. In the degenerating areas few fat droplets are seen in the cell cytoplasm.

Histogenesis.—It is now generally agreed that undifferentiated cells of mesenchymal origin are found in lymph nodes and hemopoietic organs of the adult. These cells possess a rich and varied potentiality to develop into an amazing variety of cells, which may form supporting structures (cartilage, bone and connective tissue), lymphoid, myeloid and myeloblastic tissue, and the reticuloendothelial system of phagocytic cells. In normal health these cells are inconspicuous. They are situated outside and in close apposition to the lining cells of the blood and lymph sinuses. They are pale cells; their outlines are indistinct, and they appear to form a syncytial network. The cells possess faint clear nuclei and are picked out only by their distinct nuclear membrane. They are nonmobile, do not store dyes and are not associated with argyrophilic fibrils. In response to suitable stimulation they become swollen, multiply, move out of their unobtrusive situation and assume a definite cell outline. The nuclei become indented, darker, richer in chromatin and smaller in size. The cytoplasm becomes denser, and fragments of ingested particles can be seen in it. A fine-meshed network of reticulum fibers is laid down in close association with these cells. They bear the usual microscopic characters of cells variously termed as monocytes, clasmatocytes or reticulum cells. It has been observed that the microscopic appearances, and presumably the physiologic functions, of these cells vary to a certain extent with their location and the environment in which they settle down. The limits of their determination and "the extent of their pluripotentiality" in different environments are still unsettled. It is known, for instance, that cells resembling reticulum cells can give rise to osteoblasts in birds and can revert back to their original morphologic character under certain conditions. It is also known that reticulum cells are abundantly present in normal bone marrow and can be stimulated to increased activity or even neoplastic proliferation under conditions not well understood at

present. It is, however, difficult to understand the reasons for a difference in the activity of their growth in the marrow, as compared to the rapid growth of morphologically indistinguishable cells occurring in lymph nodes and elsewhere.

Therapy.—Reticulum cell sarcoma is strikingly amenable to roentgen radiation. It has even been suggested that this marked sensitivity to radiation may be used as a diagnosgtic criterion when the tumors are inaccessible to biopsy. The usual dose of high voltage roentgen rays which produces subsidence of a tumor is about 600 r, although some lesions fail to show any evident change until after the dose has reached 800 r. In the primary reticulum cell sarcoma of bone, the improvement following irradiation seems to persist in most cases. Roentgen therapy seems to effect not only a regression of the disease but a condensation of osseous trabeculae and a regeneration of bony tissue in completely destroyed areas. This was observed in cases 1, 2 and 3 of the present report and in the tumor of the scapula described by Szutu and Hsieh. Parker and Jackson observed better results with amputation and irradiation in 8 of the 9 patients so treated. They further found that in 3 cases "in which radiation failed to arrest the disease, subsequent surgical attacks effected an eventual cure."

SUMMARY

Five cases of primary reticulum cell sarcoma of bone observed during the last six years have been described. The necessity for an early recognition of the disease and appropriate treatment of the patients has been emphasized in view of a relatively favorable course of the disease in many cases.

NEOPLASTIC DISEASES OF DOGS

II. Mast Cell Sarcoma, Lymphosarcoma, Histiocytoma

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THIS continuation of the discussion of neoplastic diseases of dogs initiated in a paper on melanoma published elsewhere [1] will include a consideration of the features of mast cell sarcoma, lymphosarcoma and histiocytoma.

REVIEW OF THE LITERATURE

Neoplasms of mast cells, of lymphoblasts and lymphocytes, and of histiocytes will be reviewed so far as they have not been adequately covered in previous papers.[2]

Mast Cell Sarcoma.—Murray [3] was the first to recognize mast cell sarcoma, although earlier workers had listed cases of "round cell sarcoma" without specifying type. He reported 2 cases in which the tumor occurred on a hindleg. Chambers [4] described mast cell sarcoma in 3 male dogs. The first tumor was on a hindleg of a 9 year old fox terrier, the second in a popliteal space of an 8 year old bull terrier and the third in an axilla of a 10 year old fox terrier. Under the name "mastocytoma," Bloom [5] recorded 5 instances of mast cell sarcoma. In 4 instances male dogs were involved, the sarcoma affecting the right thigh of a 15 year old Boston terrier, the right superior labial region of a 5 year old English setter, the scrotum, inguinal lymph nodes, spleen, liver, omentum, mesentery and lungs of a 9 year old mongrel spaniel, and the scrotum and inguinal lymph nodes of a 15 year old fox terrier. The female dog was an 8 year old fox terrier with a tumor of the left ear. The finding of heparin in mast cell sarcomas of dogs and the decrease of specific granules accompanying

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1. Mulligan, R. M.: Am. J. Path., to be published.

2. Mulligan, R. M.: (*a*) Arch. Path. **38**:115, 1944; (*b*) Cancer Research **4**:505, 1944; (*c*) Arch. Path. **39**:162, 1945; (*d*) **45**:216, 1948.

3. Murray, J. A.: Third Scientific Report, Imperial Cancer Research Fund, London, 1908, pp. 41-60.

4. Chambers, F.: Vet. Rec. **11**:709, 1931.

5. Bloom, F.: Arch. Path. **33**:661, 1942.

heightened anaplasia of these tumors have been described.[6] The characteristics of the cells of 2 mast cell sarcomas were investigated by the tissue culture method.[7]

Lymphosarcoma.—Jöhne [8] found lymphosarcoma of tracheobronchial lymph nodes. Joest observed 4 lymphosarcomas, 1 in mesenteric lymph nodes,[9a] 2 in the intestines [9b, c] and 1 generalized.[9d] Chambers [4] noted a lymphosarcoma of the pharynx of an 8 year old male golden retriever. Gaiger [10] recorded the case of a golden retriever with a lymphosarcoma involving the intestine, the mesenteric lymph nodes, the liver and one kidney. Jackson [11] included 4 cases of lymphosarcoma among 77 instances of tumors in dogs. Bloom and Meyer [12] described 13 cases of lymphosarcoma under the term "malignant lymphoma" and exhaustively reviewed the literature on neoplastic diseases of the lymphoid tissue of dogs.

Histiocytoma.—Although Auler and Wernicke [13] called this neoplasm "benign round cell sarcoma," Lacroix and Riser [14] "transmissible lymphosarcoma" and I [2d] "lymphosarcoma," closer study of more cases in this laboratory has led me to designate this tumor as "histiocytoma." Auler and Wernicke [13] described 60 cases of this type of neoplasm, which was nearly always found in the skin of young dogs, after the age of 6 months, seldom grew larger than 25 mm. in diameter, looked like a sarcoma histologically but did not recur after destruction or extirpation in any case. Of the 52 dogs observed by Lacroix and Riser,[14] 28 were less than 2 years old and 43 were younger than 5 years. The sex incidence was equal. Forty of the dogs were of four breeds: Boston terrier, Scotch terrier, cocker spaniel and fox terrier. Thirty-two tumors involved the skin of the ears, front quarters and face; only 4 tumors were located on the penis or the scrotum, and none affected the vulva or the vagina. The clinical course of the tumors was benign; many of them regressed spontaneously.[15]

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6. Oliver, J.; Bloom, F., and Mangieri, C.: J. Exper. Med. **86**:107, 1947.

7. Paff, G. H.; Bloom, F., and Reilly, C.: J. Exper. Med. **86**:117, 1947.

8. Cited by Casper, M.: Ergebn. d. allg. Path. u. path. Anat. **3** (part 2):754, 1896.

9. Joest, E.: (*a*) Ber. u. d. Königl. Tierartzl. Hochshule zu Dresden **4**:171, 1909; (*b*) **8**:75, 1913; (*c*) **14**:58, 1919; (*d*) **15-16**:42, 1920-1921.

10. Gaiger, S. H.: Vet. Rec. **11**:558, 1931.

11. Jackson, C.: The Onderstepoort Journal **6**:1, 1936.

12. Bloom, F., and Meyer, L. M.: Am. J. Path. **21**:683, 1945.

13. Auler, H., and Wernicke: Ztschr. f. Krebsforsch. **35**:1, 1931.

14. Lacroix, J. V., and Riser, W. H.: North American Vet. **28**:451, 1947.

15. Material from cases of mast cell sarcoma, lymphosarcoma and histiocytoma was contributed by veterinarians listed previously [1] and also by the following veterinarians: G. S. Bolton, W. L. Brayley, A. N. Carroll, H. C. Evers and W. B. Teller, W. K. Fauks, E. C. Jones, E. Laitinen, and G. G. Miller, Jr. Technical assistance was as indicated in the first of these publications.[1]

MAST CELL SARCOMA

Analysis of Cases.—The 47 cases of mast cell sarcoma studied have been summarized in table 1. Of the 45 dogs with mast cell sarcoma whose age was known, 5 were 2 to 5 years old, and the remaining 40 were 6 to 14 years old. The sex was known in 46 dogs; 29 were female and 17 were male. Among the 47 cases, the breeds represented included the following: terrier, 29 (Boston, 19; fox, 6; mixed, 3; Manchester, 1); mongrel, 3; shepherd, 3; setter, pointer, dachshund, cocker spaniel, 2 each, and boxer, great Dane, Chesapeake Bay retriever and poodle, 1 each. Noteworthy was the high incidence of mast cell sarcoma affecting Boston terriers; of the 19 studied, the sex of 14 was female and that of 5 male. Information concerning the location of the primary neoplasm was available in 47 cases: A posterior extremity was involved in 12; the scrotum, in 9; a flank in 4; the vulva, an axilla, an eyelid and an anterior extremity, in 3 each; a mammary gland and the neck in 2 each; an ear, the penile sheath, a lip, the perineum, the abdomen and the sternal region, in 1 each.

Gross Findings.—Of the 47 mast cell sarcomas, 43 were solitary and 4 were multiple. This group constituted over 8 per cent of the neoplasms studied in this laboratory. Ten were observed at autopsy, 36 were excised surgically, and 1 was studied at both operation and necropsy. Among the 10 cases in which autopsies were made, regional lymph nodes were involved in 7 and the spleen in 2. In 1 surgical case regional lymph nodes were affected.

The size of the primary neoplasm was known in 38 cases; it varied from 216 cu. mm. (6 by 6 by 6 mm.) to 576,000 cu. mm. (90 by 80 by 80 mm.). In 12 cases the volume was 9 cc. or less; in 20, 10 to 200 cc., and in 6, 200 to 600 cc. The primary neoplasm and overlying skin were often ulcerated. The cut section was usually firm and lobulated or whorled. The consistency was also described as fairly firm, elastic, friable, cystic or porous or spongy, focally softened or focally gelatinous. The color was variously noted as gray, pink-gray, white, gray-yellow or light tan and, in some cases, yellow streaked, red stippled or dark red mottled (fig. 1).

Microscopic Structure (figs. 2 to 6).—With the hematoxylin and eosin stain, the neoplastic mast cells of the more mature tumors, which tended to be relatively small grossly, were polyhedral and had discrete or sharply demarcated borders, although a stubby spindle shape resulted when they were caught among bundles of collagen fibrils. The cytoplasm was pale, acidophilic and abundant when compared with the size of the nucleus. Some cells showed a suggestively vacuolated cytoplasm. Most of the cells contained numerous coarse, dark blue granules, in many to the point of obscuring the nuclei. The nuclei

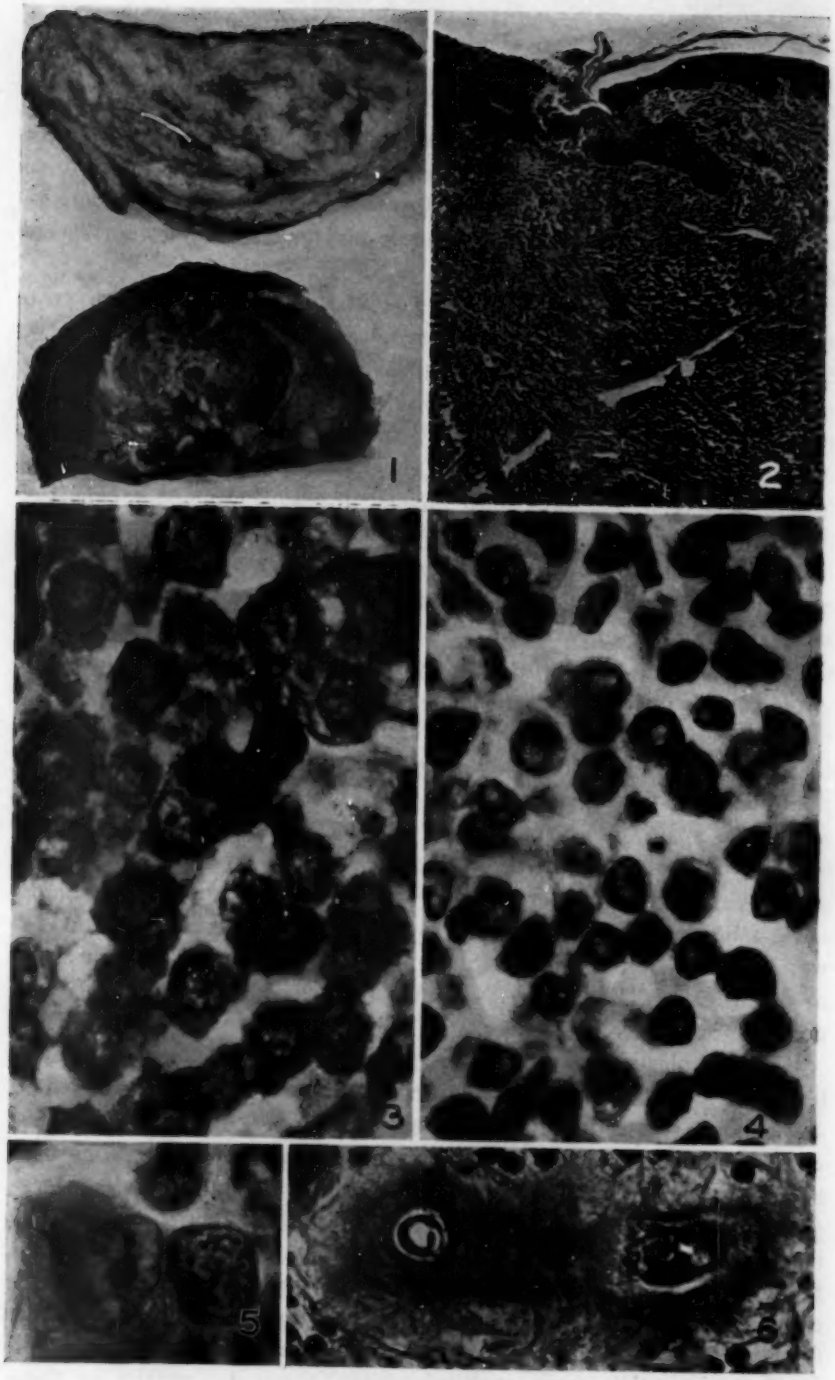
TABLE 1.—*Summary of 47 Cases of Mast Cell Sarcoma*

Case	Age of Dog, Yr.	Sex	Breed	Location
1	13	F	Fox terrier	Left flank, inguinal lymph nodes
2	9	F	Boston terrier	Left tibial region, thigh, flank
3	3	F	Boxer	Nipple, left fourth mammary gland
4	4½	F	English setter	Right tarsal region
5	12	M	Fox terrier	Left gluteal region
6	9	F	Boston terrier	Vulva
7	8½	F	Boston terrier	Right axillary region
8	10	M	Boston terrier	Scrotum
9	10	F	Boston terrier	Left gluteal region
10	12	M	Boston terrier	Scrotum, inguinal lymph nodes, spleen
11	5	F	Great Dane	Right stifle
12	8	M	Fox terrier	Left flank
13	7	M	Chesapeake Bay retriever	Scrotum
14	14	F	Mongrel terrier	Right dorsal cervical region
15	9	M	Boston terrier	Hindleg
16	14	M	Mongrel terrier	Scrotum
17	12	M	Wire-haired fox terrier	Ear
18	9	F	Pointer	Vulva
19	4	F	Poodle	Left hindleg
20	7	F	Boston terrier	Left flank
21	7	F	Boston terrier	Left flank
22	12	..	Shepherd	Axilla
23	10	M	Mongrel shepherd	Prepuce, penile sheath, inguinal lymph nodes
24	7	F	Boston terrier	Prescapular region
25	8	M	Irish setter	Hindleg, lymph nodes, spleen
26	14	M	Mongrel	Eyelid
27	Old	M	Mongrel	Scrotum
28	11	M	Boston terrier	Scrotum, penile sheath
29	10	F	Dachshund	Upper lip, regional lymph nodes
30	8	F	Boston terrier	Right eyelid
31	14	M	Boston terrier	Scrotum, inguinal lymph nodes
32	Old	F	Boston terrier	Perineum
33	10	F	Boston terrier	Left carpal region, axillary lymph nodes
34	11	F	Mongrel fox terrier	Right thigh
35	12	M	Fox terrier	Scrotum, inguinal lymph nodes
36	11	F	Dachshund	Vulva
37	7	F	Cocker spaniel	Sternal region
38	7½	F	Boston terrier	Right upper eyelid
39	14	M	German shepherd	Right thigh
40	8	F	Mongrel	Foreleg
41	11	F	Boston terrier	Mammary gland
42	13	M	Mongrel terrier	Scrotum
43	12	F	Cocker spaniel	Left forearm, axillary lymph nodes
44	10	F	Manchester terrier	Thigh
45	7	F	Pointer	Left axilla
46	2	F	Boston terrier	Right gluteal region
47	13	F	Boston terrier	Abdomen

were round or slightly oval and most often centrally located, but occasionally were eccentric. Sometimes they were dark and condensed. The perinuclear membrane was fairly heavy but discrete and uniform. The chromatin was fine, homogeneous and evenly distributed. Rare binucleated cells were observed, and mitotic figures were unusual or absent. The nuclei were fairly uniform in size and were occasionally reniform. Nucleoli were small, discrete and usually single, but rarely were double.

In the less differentiated neoplasms, studied with the hematoxylin and eosin stain, the borders of the neoplastic cells tended to be fuzzy; the cytoplasm was pale to moderately basophilic and was relatively small in volume in relation to the size of the nucleus. Sometimes the volume of the nucleus was so great as to leave but a narrow rim of cytoplasm, with the result that the cell resembled a lymphoblast. Eccentricity and multiplicity of nuclei in some cells reminded of plasmablasts. The cytoplasmic granules were pale purple, few and fine, or occasionally coarse, dark blue and fairly abundant, or, most commonly, could not be demonstrated except by Giemsa or Nissl stains. Even these two stains showed but few specific granules in the cells of two neoplasms studied. Because of the relatively few granules, the nuclei were conspicuous, rounded and enlarged to varying degrees. Some nuclei were quite irregular and warped into bizarre shapes. Giant nuclei were occasionally seen, especially in multinucleated tumor cells. Some cells contained two or more, occasionally as many as seven, nuclei. Mitotic figures were irregular and varied from a few to many. The chromatin was irregular, coarse and more abundant at the periphery of the nucleus. Especially in multinucleated tumor cells the chromatin was excessively heavy. Nucleoli were enlarged and prominent and sometimes numbered two or three to a nucleus. The features of anaplasia were definitely more pronounced in recurrent tumors.

The neoplastic mast cells were arranged in groups or were scattered singly and often were separated by irregular bundles of increased collagenous connective tissue. The proportions of neoplastic mast cells and connective tissue varied. Broad bands of collagenous connective tissue were intermingled with scattered tumor cells, or large groups of tumor cells were mixed with isolated bundles of collagen fibrils, or solid masses of tumor cells showed no related connective tissue. Locally the neoplastic cells were present within lymphatic channels and veins and invaded skeletal muscle. In lymph nodes the neoplastic cells infiltrated sinusoids, trabeculae and lymphoid cords. In the spleen they were enclosed within sinusoids, usually in those adjacent to the lymphoid nodules.



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The stroma of increased collagenous connective tissue contained scattered or many segmented neutrophils, sometimes to the point of formation of miliary abscesses. The connective tissue stroma was infiltrated less abundantly in some cases by lymphocytes and also by scattered eosinophils and plasma cells. Mature blood cells, especially neutrophils, were also mingled with tumor cells. Areas of myxomatous degeneration of the connective tissue were fairly frequent. Abundant granulation tissue mingled and merged with the connective tissue stroma near the surface of ulcerated neoplasms. Areas of edema and necrosis affected masses of neoplastic cells and stroma both in the local neoplasm and in affected regional nodes in several tumors. Heavy deposits of hyalin encompassed blood vessels in an occasional tumor.

When intact, the epidermis of the overlying skin was diffusely thinned or was thick and acanthotic in small areas. Most often the epithelium was destroyed and the ulcerated surface was covered with fibrinopurulent exudate, which blended with underlying abundant granulation tissue. In ulcerated tumors the epidermis next to the ulcers often showed increased thickness, lengthened and broadened rete pegs, acanthosis, and mitotic figures in the deepest layers. Cystic and atrophic sebaceous and sweat glands were numerous and intimately associated with the tumor and its supporting stroma in several neoplasms, thus explaining the porous, spongy or cystic appearance noted grossly.

LYMPHOSARCOMA

Seven illustrative cases are reported:

Case 1.—A spayed female Boston terrier, 9 years old, had a lymphoblastic lymphosarcoma of the cervical, tracheal, mediastinal, mesenteric and inguinal lymph nodes, the spleen, the liver, the bone marrow and the lungs. The lymph nodes ranged between 17 and 22 mm. in greatest diameter. A pink-gray nodule, fairly soft, measuring 2 cm., involved the upper pole of the spleen. The blood contained: hemoglobin, 14.2 Gm. per hundred cubic centimeters; erythrocytes,

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Fig. 1 (case 9, table 1).—Mast cell sarcoma. A cut section is shown at the top; the line of excision from surrounding tissues, at the bottom. Skin partly covers both portions. × 4/5.

Fig. 2 (case 11, table 1).—Mast cell sarcoma. An ulcerated area is seen in the left upper corner. Note the hyperplastic epidermis at the surface. The tumor pattern suggests stippling, each black dot representing a neoplastic mast cell. × 50.

Fig. 3 (case 11, table 1).—Neoplastic mast cells with many specific granules. × 1,000.

Fig. 4 (case 12, table 1).—Neoplastic mast cells with few or no specific granules. × 1,000.

Fig. 5 (case 8, table 1).—Neoplastic mast cells with giant bizarre nuclei. × 1,000.

Fig. 6 (case 22, table 1).—Mast cell sarcoma showing deposit of hyalin around a blood vessel. × 450.

(All these photomicrographs were taken from tissue sections stained with hematoxylin and eosin.)

6,250,000 per cubic millimeter; leukocytes, 14,300 per cubic millimeter, with stab neutrophils 7, segmented neutrophils 77, lymphocytes 14 and normoblasts 2 per cent.

CASE 2.—A male Scotch terrier, 9 years old, had a lymphoblastic lymphosarcoma in the cervical, mediastinal and mesenteric lymph nodes (11 to 21 mm. long), the spleen, the liver and the lungs. The bone marrow showed diffuse hyperplasia. The blood contained: hemoglobin, 11.3 Gm. per hundred cubic centimeters; erythrocytes, 5,330,000 per cubic millimeter; leukocytes, 12,050 per cubic millimeter, with stab neutrophils 17, segmented neutrophils 63, lymphocytes 18, monocytes 1 and normoblasts 1 per cent.

CASE 3.—A spayed female Scotch terrier, 9 years old, suffered from generalized lymphadenopathy, dyspnea and anorexia for seven months. Autopsy disclosed a lymphocytic lymphosarcoma in the cervical, tracheobronchial, mediastinal, mesenteric and inguinal lymph nodes, the spleen, the liver, the bone marrow, the gallbladder, the stomach, the kidneys, the lungs and the thyroid and parathyroid glands. Two agonal ulcers marked the antral mucosa of the stomach. The thyroid gland showed diffuse hyperplasia.

CASE 5.—A female collie, 3 years old, showed anorexia, loss of weight and diarrhea for three months before death. Autopsy disclosed in the first 8 cm. of the colon a diffuse pink-gray soft infiltration of the wall, so that the outside diameter of the colon was 6 cm. and the lumen was narrowed to an irregular slit. In the mesentery of the intestines masses of pink-gray soft lymph nodes measured 16 by 12 by 10 cm., and in the anterior mediastinum similar masses measured 10 by 8 by 7 cm., with single nodes 2 to 5 cm. in greatest diameter. The right lobes of the liver contained two umbilicated round pink-gray nodules, 4 and 5 cm. in diameter. The microscopic diagnosis was lymphoblastic lymphosarcoma.

CASE 6.—A male cocker spaniel, 9 years old, had anorexia, emaciation, generalized lymphadenopathy and dyspnea for two months before death. At autopsy, lymphoblastic lymphosarcoma involved the submaxillary, cervical, tracheobronchial, mediastinal, mesenteric, retroperitoneal, iliac and inguinal lymph nodes, the spleen and the liver. The cervical lymph nodes ranged to 9 cm. in length, the submaxillary to 4 cm., and the remaining nodes ranged between 2 and 7 cm. The bone marrow showed myeloid hyperplasia and was not infiltrated by specific cells.

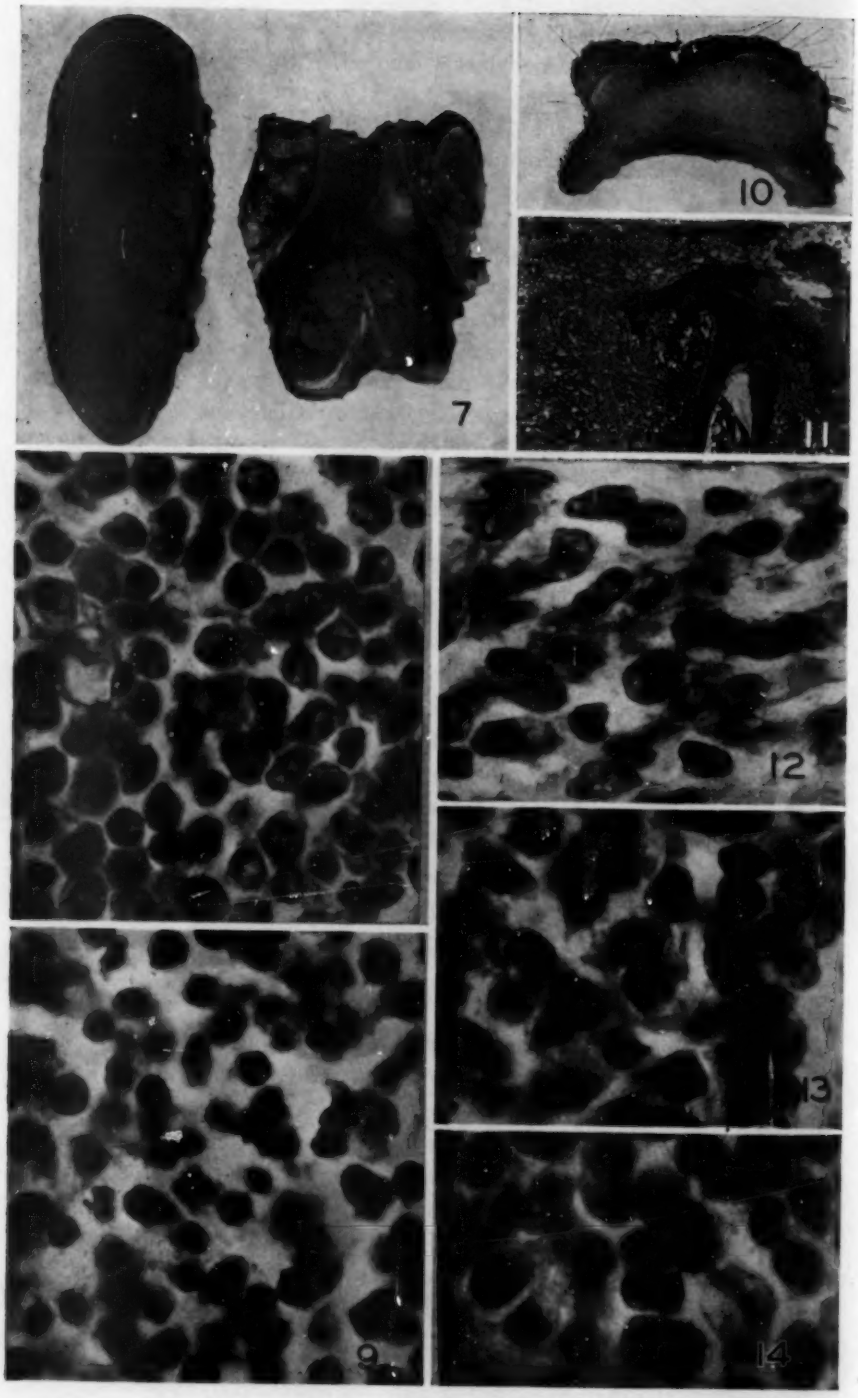
CASE 7.—A spayed female Scotch terrier, 10 years old, showed generalized lymphadenopathy, anorexia, loss of weight and respiratory distress for three months before an enlarged right prescapular node was subjected to biopsy, which established the diagnosis of lymphocytic lymphosarcoma. The blood smear showed: stab neutrophils, 5 per cent; segmented neutrophils, 40.5 per cent; lymphocytes, 45.0 per cent; eosinophils, 8.5 per cent; monocytes, 0.5 per cent, and normoblasts, 0.5 per cent. One gram of urethane U. S. P. was given intramuscularly daily for thirty-five days, but the superficial lymph nodes continued to enlarge and the symptoms progressed. Autopsy disclosed unaltered lymphocytic lymphosarcoma in the 2 to 5.5 cm. submaxillary, cervical, tracheobronchial, mediastinal, mesenteric, prescapular and popliteal lymph nodes and in the liver, the bone marrow, the kidneys, the stomach and the parathyroid glands. The Scotch terrier described in case 3 was a litter mate. A male of the same litter had similar clinical findings before death, at the age of 7 years, but no autopsy was made.

Analysis of Cases.—The 16 cases of lymphosarcoma accounted for about 2.5 per cent of the neoplasms studied in this laboratory. In 15 the age was available; 3 dogs were 3 to 5½ years and 12 were 6 to 12 years old. The sex of the 16 dogs was male in 5 and female in 11 (6 intact, 5 spayed). The following breeds were represented: terrier, 11 (Scotch, 5; Boston, 2; fox, 2; English bull, 1, unspecified, 1); spaniel, 4 (cocker, 3; English springer, 1), and collie, 1. The tissues studied were surgically excised in 4 cases, obtained at autopsy in 11, and were studied at both operation and necropsy in 1 case. Lymph nodes were involved in all 16 cases. In 12 cases all lymph nodes (submaxillary, cervical, tracheal, bronchial, mediastinal, mesenteric, retroperitoneal, iliac, axillary, prescapular, inguinal) were affected. In 4, local lymph nodes were involved, submaxillary in 2 and cervical in 2. Among the 12 cases in which autopsies were made, the spleen was involved in 9 of 10 in which it was examined, the liver in 9 of 10, the kidneys in 4 of 8, the colon in 2 of 7, the lungs in 3 of 6, the stomach in 2 of 6, the bone marrow in 3 of 5, the parathyroid glands in 2 of 5, the thyroid gland in 1 of 5 and the gallbladder in 1 of 5.

Gross Findings.—The enlarged lymph nodes ranged from 1 to 9 cm. in greatest diameter, were soft or elastic or semiliquid, and pink-gray or tan (fig. 7). The spleen was enlarged, soft, bulging on section and marked by many rounded gray areas 0.5 to 2 mm. in diameter. One spleen contained a 2 cm. pink-gray soft nodule. The liver was of normal size or moderately enlarged. The periportal areas were outlined by gray dots and irregular gray streaks. One liver showed two pink-gray soft nodules, 4 cm. and 5 cm. in diameter, in the right lobes. The stomach, the colon and the gallbladder showed diffuse or localized gray thickening of the mucosa or the wall or of both. The kidneys, the lungs, the thyroid gland and the parathyroid glands were not remarkable grossly.

Microscopic Examination (figs. 7 to 9).—Of the 16 cases studied, 14 were of the lymphoblastic type and 2 of the lymphocytic type.

With the hematoxylin and eosin stain, the lymphoblast had a polyhedral or an undulated or scalloped border and fairly plentiful polychromatophilic or pale acidophilic cytoplasm. The nuclei were usually round or oval, sometimes reniform, and often irregular. The chromatin was coarse, unevenly distributed, and clumped together most abundantly at the edge of the nucleus. The heavy nuclear membrane was sharply defined. Nucleoli were large, prominent, acidophilic, and numbered one or two, sometimes three, in a nucleus. Mitotic figures were fairly numerous. Cells intermediate in development between lymphoblasts and lymphocytes were intermingled. Mature lymphocytes were also scattered among masses of lymphoblasts.



(See legend on opposite page)

The lymphocyte was round and discrete and had a pale acidophilic cytoplasm, scanty to fairly abundant. The nuclei were round or slightly oval and sharply demarcated from the cytoplasm. The chromatin was heavy, condensed and frequently was gathered in a solid mass throughout the nucleus. In some nuclei the edges of nucleoli, largely obscured by condensed chromatin, were suggestively visible. Mitotic figures were absent. Scattered lymphoblasts were mingled with masses of lymphocytes.

With Giemsa stain, the cytoplasm of the lymphoblast was moderately to slightly basophilic, and that of the lymphocyte, pale basophilic. The nucleoli of the lymphoblast were deep basophilic.

Regardless of the type of cell proliferated, the lymph node involved showed its components rather monotonously inundated by lymphoblasts or by lymphocytes. The lymphoid nodules were absent as such, and at their former sites cells were blended imperceptibly with the lymphoid cords. The sinusoids were filled with specific cells, so that the borders between cords and sinusoids were obliterated or obscured and the node became one mass of proliferated cells. In the background were scattered lymphocytes with cytoplasmic bodies intact but with nuclei broken into deeply basophilic round fragments. In the background was noted also a fine sprinkling of reticuloendothelial cells, foamy macrophages, plasma cells, and phagocytes enclosing nuclear fragments. The capsule of the lymph node, the surrounding adipose and connective tissues, and occasionally skeletal muscle were infiltrated by specific cells.

In the spleen the lymphoid nodules were composed of specific cells, were greatly enlarged and were fused together. The red pulp was more or less infiltrated and obliterated. In the liver the periportal areas were infiltrated by specific cells and were widened to a varying degree. In the lungs the lymphoid nodules were composed of specific cells and were increased in both size and number. In the kidneys infiltrations were noted in the stroma of the cortex and in the peripelvic adipose tissue. All coats of the colon were diffusely involved. The mucosa of the stomach was affected. The specific cells widely flooded the bone

Fig. 7 (case 6).—Lymphosarcoma. A cut section of an enlarged cervical lymph node is shown at the left, and one of hypertrophied tracheobronchial lymph nodes, at the right. × 7/10.

Fig. 8 (case 13).—Neoplastic lymphoblasts from a lymph node. × 1,000.

Fig. 9 (case 3).—Neoplastic lymphocytes from a lymph node. × 1,000.

Fig. 10 (case 21, table 2).—Histiocytoma, cut section. Note the ulcerated skin surface at the top. × 1⅓.

Fig. 11 (case 21, table 2).—Histiocytoma. An ulcerated surface is shown at the top, with hyperplastic epidermis and hair follicle beneath and to right, surrounded by closely packed neoplastic histiocytes. × 35.

Fig. 12 (case 7, table 2).—Neoplastic histiocytes in skin. × 1,000.

Fig. 13 (case 19, table 2).—Neoplastic histiocytes in skin. × 1,000.

Fig. 14 (case 8, table 2).—Neoplastic histiocytes in skin. × 1,000.

(All these photomicrographs were taken from tissue sections stained with hematoxylin and eosin.)

marrow by displacing the usual granulocytic, erythrocytic and megakaryocytic elements. All coats of the gallbladder were infiltrated. In the parathyroid glands the specific cells crowded between cords of parenchymal cells. Small foci of specific cells marked the stroma between follicles of the thyroid gland. No leukocytes diagnostic of leukemia were found in the peripheral blood in the 4 cases in which it was examined.

HISTIOCYTOMA

Analysis of Cases.—Among 21 cases studied, and summarized in table 2, 7 involved dogs less than 1 year old, 7 dogs 1 to 2 years old, 5 dogs 2 to 5 years old, and 2 dogs over 6 years old. The sex was male in 15 and female in 6. The following breeds were represented: terrier, 9 (Boston, 4; fox, Scotch, American bull, Kerry blue, and unspecified, 1 each); spaniel, 8 (cocker, 7; English springer, 1), and poodle, shepherd, English setter and boxer, 1 each.

The location of 20 neoplasms was as follows: anterior extremity, 6; posterior extremity, 4; ear, face, axilla and thorax, 2 each; penile sheath and vagina, 1 each. On the extremities, the carpal and metacarpal regions, the elbow, the hock and the stifle were affected.

Gross Findings.—Without exception, all 21 tumors studied were excised surgically. They constituted about 3.5 per cent of the neoplasms examined in this laboratory. They ranged in size from 288 cu. mm. (8 by 6 by 6 mm.) to 15,246 cu. mm. (42 by 33 by 11 mm.). Three were less than 900 cu. mm. in volume, 8 were 1,000 to 1,800 cu. mm., 6 were 2,000 to 5,000 cu. mm., 3 were 8,000 to 12,000 cu. mm. and 1 was 15,000 to 16,000 cu. mm.

Grossly, the tumors were often ulcerated, firm, white or light gray, usually homogeneous, occasionally finely nodular and sometimes red stippled or dark red mottled (fig. 10).

Microscopic Examination (figs. 11 to 14).—With the hematoxylin and eosin stain, the neoplastic histiocytes had abundant, finely granular, acidophilic or polychromatophilic cytoplasm with an indefinite, undulated, wavy border and with frequent overlapping of adjacent cells. The nuclei were usually round or oval but were also oblong, reniform, C shaped, wrinkled or even shapeless. The nuclei varied considerably in size. Occasional binucleated forms were observed. The nuclear membrane was thin and distinct. The evenly distributed chromatin was threadlike or filamentous or finely beaded. The nucleoli were small, acidophilic, usually single and occasionally double. Many regular mitotic figures were seen. In some nuclei the chromatin was partly condensed and shifted toward the nuclear periphery; nucleoli were enlarged and prominent, numbering two or three in some nuclei. Such nuclei were quite plentiful in one tumor which contained many small

areas of coagulation necrosis. The neoplastic cells infiltrated widely throughout the dermis and hypodermis and frequently crowded around hair follicles, sebaceous glands and sweat glands. Bundles of collagen were intermingled with masses of tumor cells. Scattered lymphocytes and occasional mast cells, plasma cells and segmented neutrophils were also interspersed among the tumor cells. In some tumors, lymphocytes were present in solid aggregates or in palisaded rows. The borders of the tumors merged indefinitely with surrounding, unaffected connective tissue stroma. A few small areas of coagulation necrosis marked some tumors.

TABLE 2.—*Summary of 21 Cases of Histiocytoma*

Case	Age of Dog, Yr.	Sex	Breed	Location
1	12	M	Boston terrier	Right thoracic region
2	3½	F	Cocker spaniel	Left metacarpal region
3	12	M	Cocker spaniel	Left carpal region
4	15	F	Cocker spaniel	Right axillary region
5	24	M	Scotch terrier	Right metacarpal region
6	60	F	Cocker spaniel	Left ear
7	7	M	American bull terrier	Left stifle
8	48	M	Kerry blue terrier	Left foreleg
9	4	F	Cocker spaniel	Right axilla
10	48	M	Poodle	Prepuce
11	3	M	Mongrel shepherd	Left ear
12	4	M	Cocker spaniel	Skin
13	24	M	Boston terrier	Right hock
14	7	M	Cocker spaniel	Left pectoral region
15	108	F	Boston terrier	Left elbow
16	12	M	Boxer	Supraorbital region
17	18	M	Boston terrier	Right carpal region
18	5½	M	English setter	Left hock
19	96	M	Fox terrier	Left buccal region
20	18	F	English springer spaniel	Vagina
21	18	M	Terrier	Left stifle

With Giemsa stain, the cytoplasm of the neoplastic histiocytes was pale to moderately basophilic but contained no granules. The nucleoli were basophilic. With Foot's modification of Bielschowsky's stain, reticulum fibrils surrounded groups of tumor cells but were not intimately associated with single cells, an observation indicating that these fibrils were present only as part of the supporting stroma and were not formed by the cells themselves. With sudan IV stain, the tumor cells did not contain tingible lipid. This tended to show that the neoplastic cells may have been stimulated to proliferation by antigens rather than by fatty substances infiltrating their cytoplasm. The cells of the 19 extragenital neoplasms were identical with those of the 2 observed in the penile sheath and the vagina.

In addition to reticulum, the stroma consisted of varying amounts of collagenous connective tissue. Areas of edema were noted in some tumors.

The epidermis was often ulcerated and replaced by a crust of fibrinopurulent exudate, which was focally necrotic and marked by masses of bacteria, seen as cocci with Giemsa stain. Granulation tissue was minimal or absent at the ulcerated surface. When intact, the epidermis sometimes was thinned, and the rete pegs were flattened; at other times the intact epidermis, thrown into broad folds, had a thick corneal layer and showed acanthosis, elongated and broadened rete pegs and scattered mitotic figures. Sometimes vesicles and pustules marked the epidermis.

DEFINITION OF TERMS

The term "sarcoma," deeply ingrained in the literature is suitable for labeling any cancer of mesenchymal origin, even though it means simply "fleshy tumor." Since mesenchyme is generally accepted as the parent tissue of mast cells, lymphoblasts and lymphocytes, the term "mast cell sarcoma" has been used to designate neoplasms of mast cells, and the term "lymphosarcoma," neoplasms of the cells of the lymphoid series.

"Mast cell sarcoma" is an appropriate name for the neoplastic growths of mast cells by priority,[3] if for no other reason. Murray [3] found that some mast cell sarcomas were "very malignant" but that others were "practically benign." Bloom [5] also observed variation in the degree of "malignancy" of neoplasms of mast cells. Microscopic gradations of anaplasia have been observed in the neoplasms of mast cells in the 47 cases described. The rapid growth of these tumors, which usually attain large dimensions in a few months, although they may have grown relatively slowly for as long as a year previously; the difficulty of defining the limits of a given tumor at operation so that enough tissue is resected to ablate it; the strong tendency to recur (one to three times); the increasing size and wider spread of the tumor with recurrence; the heightened signs of anaplasia of the neoplastic mast cells in the recurrent lesions; the presence of multiple tumors in about 8 per cent of cases, and the proved involvement of regional lymph nodes in over 15 per cent of cases—all these features argue in favor of "mast cell sarcoma" as the name most suitable for designating neoplasia of mast cells.

A variety of names has been applied to the group of neoplastic expressions of the cells of the lymphoid series, such as "pseudoleukemia," "aleukemic leukemia," "lymphoblastoma," "lymphomatoid diseases" and "malignant lymphoma." The term "lymphosarcoma" most adequately encompasses this group whether the origin is local or diffuse in the lymphoid tissues and whether or not diagnostic cells are found in the

peripheral blood. Thus, the cancers of the cells of the lymphoid series, grouped under the basic name "lymphosarcoma," may be divided into stem cell (reticulum cell, reticuloendothelial cell), lymphoblastic, lymphocytic and giant follicular or nodular types. The findings of only lymphoblastic and lymphocytic types of lymphosarcoma in the 16 cases described does not necessarily indicate that other types will not be observed with the examination of more cases. That lymphosarcoma is as cancerous as any neoplasm, if not the most cancerous of all neoplasms, found in the dog was proved by a total clinical course of only two to seven months, during which the affected animals showed inappetence, loss of weight and strength, respiratory distress, anemia and terminal exhaustion.

The three basic cells widespread throughout the connective tissues of the body are the fibroblast, the mast cell and the histiocyte. Even though the cells of the lymphoid series have a wide distribution, the skin is not a primary site of their origin. When found in the skin, they are cells of inflammatory or neoplastic character which circulate in the peripheral blood and which migrate from the blood vessels into the cutaneous tissues. Therefore, when the skin, sometimes that of the genitalia, of young dogs acquires a small neoplasm that develops rapidly (two to six months), is clinically benign and histologically active (such a neoplasm as has been called "benign round cell sarcoma," "benign lymphosarcoma," "transmissible lymphosarcoma" and "extravenereal lymphosarcoma"), doubt arises as to whether the neoplasm actually consists of cells of the lymphoid series, for its clinical course is quite innocent, in striking contrast to that of the fatal lymphosarcoma. This noncancerous cutaneous, sometimes genital, neoplasm consists of cells with the distinctive features of neoplastic histiocytes and has been called "histiocytoma." Since the cells of the cutaneous tumors were identical with those observed on the genitalia, the evidence supports the idea that the much debated "venereal sarcoma" previously discussed [2c] may be simply "histiocytoma." Indeed, other investigators [14] have thought that the cutaneous and genital neoplasms they examined were identical histologically, although they used the name "transmissible lymphosarcoma."

SUMMARY

Mast cell sarcoma is a neoplasm characterized as follows: 1. It occurs in dogs usually 6 years of age or older. 2. Its incidence is greater in female dogs. 3. It shows a predilection for the Boston terrier, especially the female of the breed. 4. It has a tendency to involve the extremities (usually the hindlegs), the scrotum, the flanks, the vulva and the eyelids. 5. It reaches a size of 10 cc. or more in about 70 per cent of cases. 6. Its growth is rapid; it is poorly delineated from surrounding tissues; there is a strong tendency for it to recur after excision; frequently the surface is ulcerated; the cut section is

firm, lobulated, whorled, gray, pink-gray or white; there is multiplicity of growth in about 8 per cent of cases and involvment of regional lymph nodes in over 15 per cent of cases. 7. There is anaplasia of the constitutent neoplastic mast cells as exemplified by size, number and ease of staining of specific granules, the ratio of nuclear size to cytoplasmic volume, the variation of the size and the number of nuclei, the multiplicity of nucleoli, the chromatin pattern and mitosis. 8. There is increased anaplasia in recurrent neoplasms.

Lymphosarcoma is a neoplasm which occurs usually in dogs 6 years of age or older; is perhaps more common in male and spayed female dogs; has a tendency to affect most often the Scotch terrier and the cocker spaniel; has a swiftly developing course and a fatal termination; always involves lymph nodes, often the spleen and the liver and less commonly other organs and tissues; attains large and symptom-producing size most frequently in lymph nodes; consists of lymphoblasts or of lymphocytes with characteristic cytologic features, and rarely gives rise to leukemic manifestations.

Histiocytoma is a neoplasm found predominantly in dogs 2 years of age or younger; is apparently more common in males; seems to favor the cocker spaniel; is usually located on the extremities, the face and the thorax, but seldom on the genitalia; is less than 5 cc. in volume in over 80 per cent of the cases; is acquired; grows rapidly, does not recur after excision and may regress spontaneously; is firm, white or gray and often ulcerated, and consists of neoplastic histiocytes with hazy borders, fairly abundant cytoplasm and nuclei of varying shape, which display homogeneous fine chromatin, small nucleoli and frequently mitotic figures.

Case Reports

FATAL VIRAL HEPATITIS COMPLICATED BY PHLEGMONOUS CECITIS AND ILEOCECAL INTUSSUSCEPTION

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BETHESDA, MD.

ALTHOUGH intestinal complications of acute hepatitis are not uncommon, reports in the medical literature are scant. Bergstrand [1] described phlegmonous inflammation of the intestine complicating "yellow atrophy" of the liver. Pollack and Gerber [2] encountered suppurative inflammation of the intestine in 2 of 26 cases of "acute or subacute yellow atrophy" of the liver. Lucké and Mallory [3] reported a 15 per cent incidence of phlegmonous intestinal inflammation in their series of 125 fatal cases of epidemic hepatitis.[4] In each case there was associated ascites. Pollack and Gerber found this complication in other primary diseases of the liver, such as Laennec's cirrhosis or cholangiolitic cirrhosis, but in no disease without hepatic dysfunction. Mallory and Lucké found no instance of suppurative intestinal inflammation complicating fulminant epidemic hepatitis. The incidence appears highest in the cases of the subacute hepatic infections.

While the most striking intestinal complication is phlegmonous inflammation, there are others. Hemorrhages of varying size in the intestinal wall and mesentery, noninflammatory edema of the small and large intestine and changes in the lower third of the esophagus have been reported.[4] The esophageal findings are similar to those described by Bartels as acute ulcerative esophagitis.[5]

I have been unable to find any report of intussusception complicating hepatitis. A case exhibiting this feature is herewith presented.

REPORT OF A CASE

A 60 year old white man was admitted to the United States Navy Hospital, Bethesda, Md., in a comatose state. His past history revealed that he had been

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This article has been released for publication by the Division of Publications of the Bureau of Medicine and Surgery of the United States Navy. The opinions and views set forth are those of the writer and are not to be considered as reflecting the policies of the Navy Department.

1. Bergstrand, H.: Ueber die akute und chronische gelbe Leberatrophie, Leipzig, G. Thieme, 1930.

2. Pollack, A. D., and Gerber, I. E.: Arch. Path. **36**:608, 1943.

3. (*a*) Lucké, B.: Am. J. Path. **20**:471, 1944; (*b*) Mil. Surgeon **99**:89, 1946. (*c*) Mallory, T.: J. A. M. A. **134**:655, 1947.

4. Lucké, B., and Mallory, T.: Am. J. Path. **22**:867, 1946. Lucké.[3a,b] Mallory.[3c]

5. Bartels, E. C.: Arch. Path. **20**:369, 1935.

hospitalized earlier in the same year with the diagnosis of chronic peptic ulcer for which a partial gastrectomy was performed three months prior to his present admission. During this period of hospitalization he received a total of 900 cc. of pooled blood plasma intravenously (one hundred and twenty days before the onset of symptoms).

Following discharge, he was said to have enjoyed comparatively good health except for mild epigastric distress. This increased in severity during the few weeks preceding his final entry. On the day prior to admission he complained that he "felt extremely ill." It was noted that his skin was jaundiced, and later that day he lapsed into coma. There was no history of ingestion of toxic substances or of diarrhea, chills or fever.

At the time of admission examination revealed a comatose man who appeared considerably older than his stated age. His temperature was 96 F.; pulse rate, 120; respiratory rate, 20; blood pressure, 90 systolic and 70 diastolic. The skin and the scleras were icteric. The heart and the lungs were within normal limits. The area of hepatic dulness to percussion was less than normal. No abdominal masses were palpable, and rectal examination revealed nothing of significance. The results of neurologic examination were likewise unremarkable.

Urinalysis gave negative results. The red blood cell count was 4,400,000, with hemoglobin 90 per cent. The white blood cell count was 9,200, with a normal differential count; the blood urea nitrogen was 17 mg. per hundred cubic centimeters; the carbon dioxide–combining power, 37 volumes per cent; the blood sugar, 31 mg. per hundred cubic centimeters (this rose to 250 mg. per hundred cubic centimeters after intravenous administration of 75 Gm. of dextrose and remained elevated). The icteric index was 94; the van den Bergh test for bilirubin showed 13.4 mg. per hundred cubic centimeters. The cerebrospinal fluid was shown to be normal by chemical, cytologic and bacteriologic tests. An electrocardiogram indicated that the heart's action was within normal limits.

The patient became spastic, with bilateral Babinski reflexes, increased deep reflexes and absence of abdominal reflexes. Ankle and knee clonus were elicited. Despite intravenous injection of calcium gluconate, parenteral administration of penicillin, nikethamide and oxygen and other supportive measures, the patient died within seven hours after admission.

Autopsy (thirteen hours after death).—The body was that of a poorly nourished, aged-appearing white man with definite jaundice of skin and scleras.

The serosal membranes showed petechial hemorrhages.

The liver weighed 870 Gm. and measured 17 by 22 by 5 cm. The capsule was wrinkled and the consistency decreased. The surface was smooth and light brown. The cut surface presented a mottled, reddish yellow appearance, suggesting somewhat the "nutmeg liver" of chronic passive congestion. The biliary tract and the gallbladder were not remarkable.

The peritoneal cavity contained 350 cc. of cloudy yellow fluid.

The entire gastrointestinal tract was edematous. The terminal 15 cm. of the ileum was telescoped into the cecum and the ascending colon. This was reduced with difficulty because of the extreme edema of the involved segments. The freed portion of the ileum exhibited edema and minimal fibrinopurulent exudate on the serosal surface. The cecum disclosed striking edema of all its layers, with the wall measuring 13 mm. in thickness. Despite hemorrhagic edema, the mucosa did not appear ulcerated. The mesentery and the mesocolon were slightly swollen but showed no evidence of infarction. Neither the portal venous system nor the mesenteric arteries were occluded.

The spleen weighed 100 Gm. and presented a normal color, structure and consistency.

The heart weighed 300 Gm. The coronary arteries showed calcified atherosclerotic plaques. The myocardium was dark brown and streaked with thin white strands. The lungs weighed 670 and 780 Gm., respectively, and were emphysematous, moderately edematous and congested. Their cut surfaces were brown to red, and crepitation was diminished. Slight subapical fibrosis was noted bilaterally.

The kidneys weighed 210 and 215 Gm., respectively. The capsule stripped with ease, revealing a finely granular surface with a few deep cortical scars. The cortex was narrowed, and the arteries were prominent. Corticomedullary differentiation was distinct. The cortex was pale, while the pyramids were deeply congested. Miscellaneous additional findings included a nodular thyroid gland, nodular hyperplasia of the prostate, scarring and deformity of the glans penis, bilateral testicular atrophy hypospadias, emaciation, multiple tattoo impregnations of the skin, generalized osteoporosis and edentulism. Examination of the brain was not permitted.

Microscopically, the liver showed a combination of severe necrosis and inflammation. There was extreme, irregular central necrosis with relatively few surviving hepatic cells. Those which persisted showed advanced cloudy swelling, degenerative fatty change and necrosis. Proliferation of bile ducts was especially pronounced. Foci of inflammatory cells were present throughout the portobiliary spaces. Some of these were neutrophilic granulocytes, but the majority were lymphocytes, plasmacytes and histiocytes. Many of the last contained brown pigment. Bile thrombi were numerous. No definite evidence of hepatic cell regeneration was detected. The gallbladder was not remarkable.

Sections through the cecum revealed extreme edema. There was an acute purulent exudate which extended through all the layers, with a fibrinopurulent exudate on the serosal surface. The veins and the capillaries were uniformly dilated and congested. A purulent endophlebitis was noted in the serosal layer. Sections through other portions of the large bowel as well as through the small intestine failed to disclose any changes other than edema.

The myocardium showed increased lipochrome pigment and diffuse fibrosis. Atherosclerosis was present in the coronary arteries as well as in the aorta.

The lung revealed acute exacerbation of chronic passive congestion. In addition, there were emphysema, patchy atelectasis and mucopurulent tracheobronchitis. Focal intra-alveolar hemorrhages were also present.

The kidneys disclosed evidence of biliary nephrosis as well as arterial and arteriolar sclerotic nephropathy. Subacute to chronic purulent cystitis was found. The prostate exhibited nodular glandular hyperplasia with chronic purulent prostatitis and prostatic urethritis. There was advanced atrophy of the testes. A mesenteric lymph node had undergone inflammatory hyperplasia.

The final primary pathologic diagnosis was: homolygous serum hepatitis; phlegmonous cecitis, with ilocecal intussusception.

This case illustrates not only phlegmonous intestinal inflammation, similar to that described by the authors mentioned, but also complicating intussusception. The edematous cecum acted as intussuscipiens, and a portion of the terminal ileum, as intussusceptum. Although neither process was of long standing, it is probable that the inflammatory changes in the cecum antedated the intussusception. The difficulty of reduction and the absence of multiple similar intestinal invaginations seem to indicate that the process was not agonal.

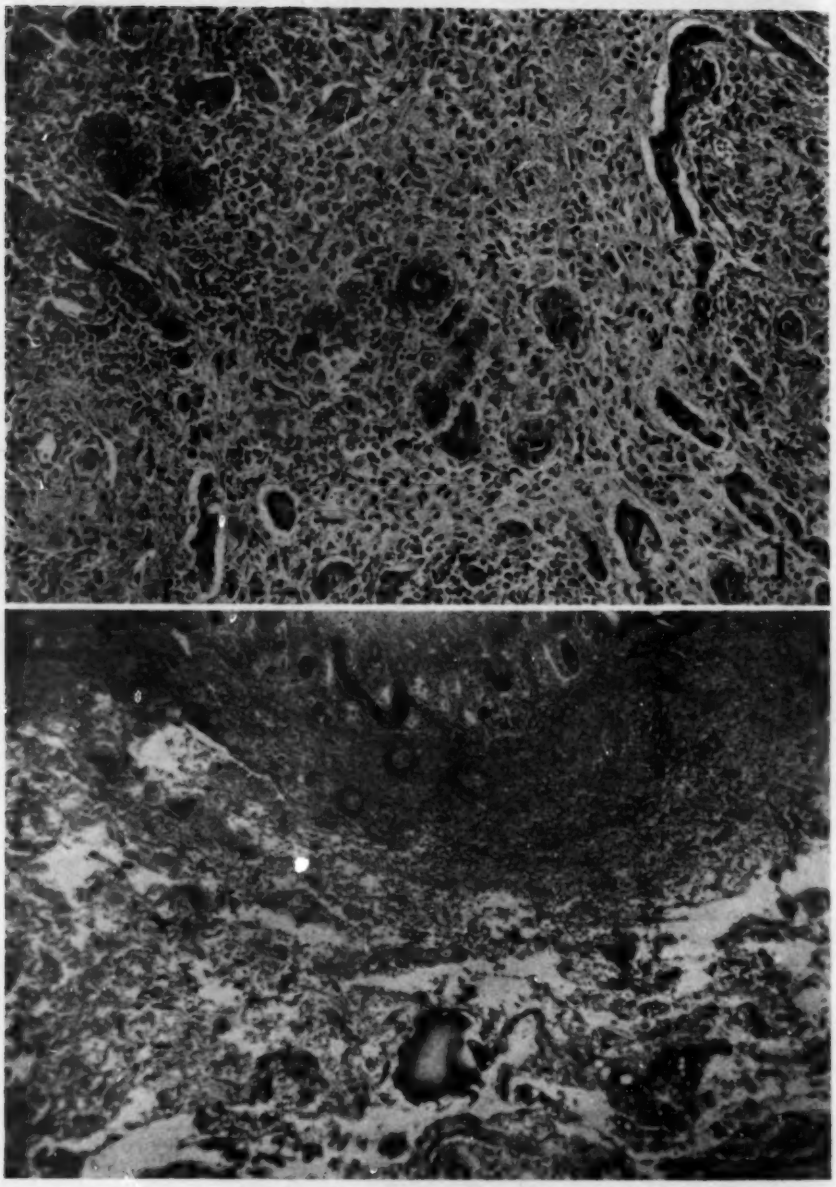

Fig. 1.—Homolygous serum hepatitis. The liver shows severe necrosis and proliferation of bile capillaries at the periphery. A few remaining hepatic cells are seen. Note the bile thrombi in the canaliculi. Note scattered acute and chronic inflammatory cells.

Fig. 2.—Phlegmonous cecitis. This section through the cecum shows severe edema and leukocytic infiltration.

It is likely that the genesis of this phlegmonous inflammation is essentially similar to that of acute ulcerative esophagitis. In the latter the important factors are decreased resistance of tissues, due to impairment of the blood supply, plus the erosive action of hydrochloric acid. In the cecum, however, it is possible that the important factors are decreased resistance of tissues and a bacterially contaminated organ

Laboratory Methods and Technical Notes

METHOD FOR BIOPSY OF BONE MARROW OF EXPERIMENTAL ANIMALS

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AND
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A TECHNIC to obtain pieces of intact marrow of sufficient size for histologic examination is as useful in experimental studies of animals as in clinical studies of anemias. Such a technic is of value also in obtaining complete hematologic data on the marrow while an experiment is in progress or prior to treatment of an experimental anemia.

The true status of the cells of the marrow cannot be shown if the death of the living tissue precedes fixation by more than a very few minutes. The problem of the study of marrow lies in the fact that the desired cells are enclosed within a heavily calcified structure which must be penetrated by the fixative as quickly as possible. The usual method of handling sections of marrow includes use of a decalcifying agent that will penetrate the bone along with the fixative. In some instances an adequate degree of fixation may be a matter of weeks, with consequent loss of staining power, due to postmortem changes in the marrow cells.

Mayer and Ruzicka [1] devised an ingenious method of exposing the marrow of a bone of a rat or a guinea pig within five minutes after the bone had been removed from the dead animal. The principles of their technic are herein applied to biopsies of the marrow of the bones of living animals.

The technic has been used on the femur of the rat for the most part. Biopsies have been made successfully on rats as young as 7 days of age. Young chicks, guinea pigs and rabbits have been used on occasion.

No aseptic precautions have been taken in the biopsies of rats and chicks, since it is well known that these animals are not exceedingly susceptible to infections. There have been no complicating infections at the sites of operation, although an occasional stitch abscess has been encountered. These have involved only the surface and subcutaneous layers of the skin in treated animals. Normal animals have shown no infections. Within the period of one week the bone is completely closed over with healthy granulation tissue. No loss of motion or of use of the bone has been seen.

The amount of marrow removed at biopsy can be controlled by the size of the incision made in the bone. There is little bleeding, and that only from the periosteal vessels. The marrow is removed intact

From the Lederle Laboratories Division, American Cyanamid Company.

1. Mayer, E., and Ruzicka, A. Q.: Anat. Rec. **93**:213, 1945.

and with little, if any, disturbance of cellular arrangement and topographic appearance, or contamination of peripheral blood. Small pieces of marrow can be obtained for smears simultaneously. Controlled experiments have shown that the biopsy has no effect on the marrow remaining in the intact bones.

The specimen is placed in the fixative immediately. In our hands the use of a neutralized alcohol–formaldehyde fixative permits the use of Graham's peroxidase stain on the tissue *en bloc*.[2] This does not interfere with the use of any routine stain, and it allows the recognition of the granulocytic series of cells within the accepted limits of the peroxidase reaction.

The technic can be used with other animals if certain modifications, including asepsis and variation of the sizes of drills, are employed.

TECHNIC

Materials Required.—The following articles should be at hand:

Anesthetic ether and paper ether cone

1 pair of medium straight-blade scissors

2 pairs of straight fine forceps

1 piece of copper wire—6 inches long, ⅛ inch in diameter (15 cm. long, 3 mm. in diameter)

Suture thread and needle or "Just Rite" skin clips

Dremel Moto-Tool Model 2 [3]

Drills—steel cutter with elongated, serrated blade—catalog nos. S-111, 112, 113 and/or S-93, 94 and 95.

Method.—1. Light ether anesthesia is maintained in the rat by means of an improvised paper cone. The relaxed animal is placed on the table on its back with its right side parallel to the edge of the table if a biopsy of the right femur is to be made. The complete operation can be done by one person when the rat, the chick or the guinea pig is used. Maintaining the anesthesia of a larger animal may require the assistance of a second person.

2. An incision is made in the skin parallel to the direction of the femur, the knife cutting laterally toward the median line. The incision need not be more than ½ to ¾ inch (about 1 to 2 cm.) in length, since the skin will stretch considerably.

3. The fat, subcutaneous tissues and blood vessels are spread free from the skin with a pair of fine forceps. This allows greater flexibility of the opening. At this point it is necessary to place one hand under the body of the animal so that manipulation may be made for changes in angles at the following steps. The thumb and the forefinger can be used as an added anchor for the dissected tissues when the drill is used in step 6.

4. The muscles over the femur are thus exposed. The closed blades of the forceps are inserted into the area of joining on the distal end of the femur. The forceps are then pushed along the line of cleavage, thus separating the two muscles and exposing the femur. By gripping the bone between the open blades of the forceps, one can strip the muscles clean from the femur by means of the

2. Ritter, H. B., and Oleson, J. J.: Arch. Path. **43**:330, 1947.

3. This is supplied by the Dremel Manufacturing Company, Fourteenth and Clark Streets, Racine, Wis.

forceps. Care must be taken to free the muscles from the distal end of the knee joint to insure flexibility of the femur for the next step.

5. One blade of the fine forceps is inserted under the distal end of the femur as close to the joint as possible and is pushed between the muscle and the bone to the other side; thus the bone is raised out of the tissues. A piece of heavy copper wire is inserted in the same manner at the proximal end of the femur. This serves to lift the bone up and to hold the tissues down and back out of the way. The femoral artery and vein run parallel to the bone at this point but can be held with the wire if the binding tissues are loosened. The femur is scraped lightly to clean away the remnants of tissue with the blades of the forceps.

6. Starting at the distal end close to the joint and extending along the shaft of the femur for the desired distance, the drilling of the bone is carried on evenly along the length of the anterior surface so that no one section of marrow is exposed before any other. The size of the drill to be used in the moto-tool[8] depends on the size of the bone and its hardness. The length of the opening depends on the amount of marrow desired. The heat of the drill does not penetrate into the bone shaft if the drilling is done quickly. With practice one can expose the endosteum without puncturing through to the marrow. Bleeding at this point can be controlled by putting pressure on a piece of cotton held to the area.

7. The endosteum is lifted away with the fine forceps, exposing the marrow. With blades closed, the forceps is inserted vertically at the shaft end of the opening. The marrow is then scooped out by tipping the forceps toward the knee joint.

8. The marrow removed is placed directly in the fixative by shaking the forceps in the bottle of fixative. Further pieces of marrow close to the joint or at the end of the shaft opening may be obtained for smears.

9. The muscles are tucked back into place after the bone levers have been removed. The fat and subcutaneous tissues are shifted into approximately their former position. The skin is closed with interrupted sutures or skin clips. At this point the animal is usually out of the ether and will begin to walk around immediately. The clips may be removed in a few days.

Samples of marrow can be obtained from living animals by this biopsy technic. The quantity of marrow obtained permits a histologic study of marrow at any point in an experiment. The biopsy material can be used as a control for changes following further treatment of the same animal. With the larger animals, the procedure suggests that repeated biopsies may be made at stated intervals. With the smaller animals, one intact bone is available for terminal study.

SUMMARY

A technic is offered for biopsy of the marrow of the living young animal. The rat, the guinea pig, the rabbit and the chicken have been used.

Notes and News

Appointments.—R. Schrek, chief of the tumor research unit of the Veterans Administration Hospital at Hines, Ill., has accepted appointment as assistant professor of pathology in the Northwestern University Medical School, Chicago.

J. L. Arteta was recently appointed professor of histology and pathologic anatomy of the Faculty of Medicine of Santiago de Compostela, Spain. He is of the school of Cajal.

Harry L. Clark, after having been associated with Wayne University, Detroit, nearly forty years, is now emeritus professor of bacteriology and clinical pathology. He joined the faculty in 1919 and has held the rank of professor of bacteriology and clinical pathology since 1922.

J. M. Ravid, formerly pathologist at the Israel Zion Hospital, Brooklyn, has been appointed pathologist to St. Clare's Hospital, New York.

Edward A. Gall, assistant professor in the University of Cincinnati College of Medicine, has been appointed Mary M. Emery professor of pathology and head of the department, also director of pathology in the Cincinnati General Hospital. He succeeds the late R. S. Austin.

Alan R. Moritz has been appointed professor of pathology in the Western Reserve University School of Medicine and director of pathology in the University Hospitals of Cleveland. At present Dr. Moritz is professor of legal medicine at Harvard Medical School and pathologist-in-chief at Peter Bent Brigham Hospital, Boston. In his new post he will succeed Howard T. Karsner, retired, who has been appointed Medical Adviser to the Surgeon General, United States Navy.

Cushman D. Haagensen, coordinator of cancer teaching at Columbia University College of Physicians and Surgeons, has been named director of the cancer institute of the Columbia-Presbyterian Medical Center. Dr. Haagensen is also assistant attending surgeon and assistant surgical pathologist at the Presbyterian Hospital, New York.

New Laboratories.—The Public Health Research Institute of the City of New York has completed preliminary work toward setting up a laboratory for determining the Rh factor in human blood. Another laboratory recently developed performs virus diagnosis for physicians free of charge. Both laboratories are operated by the Bureau of Laboratories of the Department of Health.

A virus laboratory is to be set up by the University of Buffalo at Buffalo General Hospital. To help with its organization, Gilbert Dalldorf, Albany, director of the Division of Laboratories and Research in the State Department of Health, has accepted appointment as visiting professor of pathology at the medical school.

Books Received

HEMATOLOGY. By Cyrus C. Sturgis, M.D., professor of internal medicine and chairman of the department of internal medicine of the University of Michigan Medical School, and director of the Thomas Henry Simpson Memorial Institute for Medical Research of the University of Michigan. Pp. 915, with 81 illustrations. Price $12.50. Springfield, Ill.: Charles C Thomas, Publisher, 1948.

This book deals with the diseases of the blood in 21 substantial chapters. Technical methods are not described except that chapters 22 and 23 deal with sternal puncture and with transfusion, respectively. There are 9 colored plates illustrating the blood affected by various diseases; all hematic preparations were stained by Wright's method and magnified 960 times and are well described. Of the 71 black and white figures, many are charts, some of which are not easy to read on account of the small print. This is true also of some of the tables. The text summarizes present knowledge of the diseases of the blood with special reference to the historical development of such knowledge. Each chapter contains a section on the history of the disease in question. The bibliography contains 1,830 carefully selected items. Numbered in the text, the references are placed at the bottoms of the pages, and there is also an alphabetical list (50 pages) at the end of the book. In some cases German nouns in the references are not capitalized. The book contains a vast amount of reliable information about blood diseases and will be of great help to physicians and advanced students. There is a tendency to prolixity, which should be controlled in future editions.

COLLECTED PAPERS OF THE MAYO CLINIC AND THE MAYO FOUNDATION. Edited by Richard M. Hewitt, M.D.; A. B. Nevling, M.D.; John R. Miner, Sc.D.; James R. Eckman, Ph.D.; M. Katharine Smith, B.A.; Carl M. Gambill, M.D., M.P.H.; Elizabeth L. Skafte, B.A., and Florence Schmidt, B.S.E. Volume 39, 1947. Pp. 871, with 128 illustrations. Price, $12. Philadelphia and London: W. B. Saunders Company, 1948.

NEUROSURGICAL PATHOLOGY. By I. Mark Scheinker, M.D., assistant professor of neuropathology and assistant professor of medicine (neurology), University of Cincinnati College of Medicine; neuropathologist and attending neurologist, Cincinnati General Hospital. Pp. 370, with 238 illustrations. Price $8.75. Springfield, Ill.: Charles C Thomas, Publisher, 1948.

Almost half of the volume deals with injuries of the central nervous system. The first chapter considers cerebral swelling, a term introduced to denote the gross appearance of a brain with local or diffuse increase in bulk of one or both hemispheres. Cerebral swelling in this newly assigned meaning is characterized by tumefaction, edema and liquefaction, different stages of the same process. The alterations following injuries of the central nervous system are presented in a sequential manner, the text giving a moving rather than a static picture of the processes. Though preference is given to the author's own concepts, documented with appropriate case reports, references to historical and experimental data are not wanting. In the chapter dealing with neoplasms of the central nervous system it is emphasized that most of them may be recognized in preparations stained with hematoxylin and eosin. Special staining methods are of little help in the recognition of rare tumors which cannot be classified by routine examination. The modified classification of the neoplasms is simple and adequate for that purpose. The last two chapters deal with cerebral abscess and hydrocephalus. A bibliography with key references and an index conclude the volume. The illustrations are good and well reproduced, and the print is easy to read.